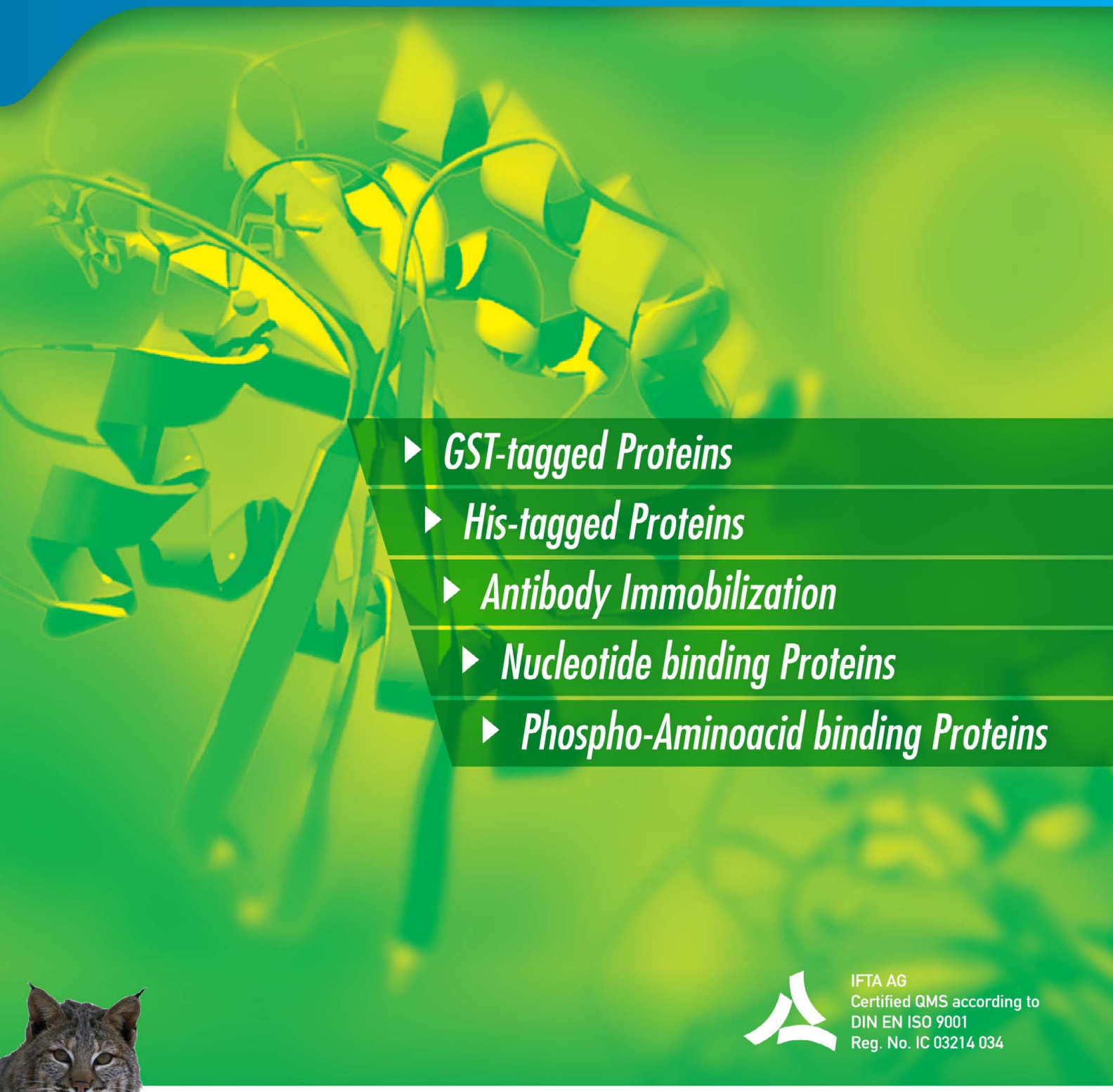
Affinity Chromatography



- GST-tagged Proteins
- His-tagged Proteins
- Antibody Immobilization
- Nucleotide binding Proteins
- Phospho-Aminoacid binding Proteins

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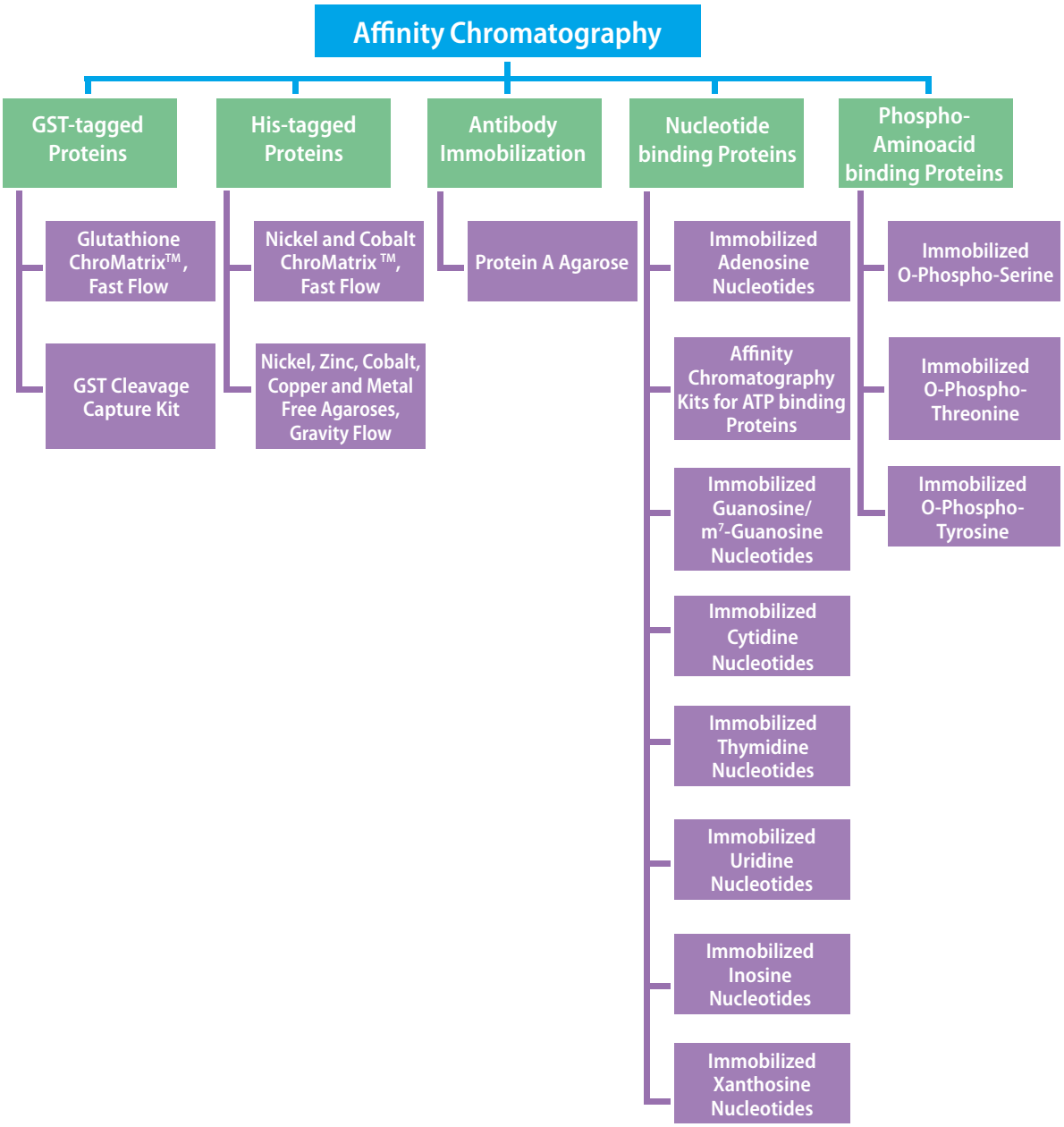
Affinity Chromatography

The ability to purify recombinant proteins using affinity chromatography has greatly advanced protein research.

In affinity chromatography, the protein of interest is purified by its ability to bind a specific ligand that is immobilized on a chromatographic bead material (matrix). This matrix is usually packed into a column.

Crude cell-lysates are loaded onto the column under conditions that ensure specific binding of the protein to the immobilized ligand. Other proteins that do not bind the immobilized ligand are washed through. Elution of the bound protein of interest can be achieved by changing the experimental conditions to favour desorption.

Jena Bioscience offers a wide range of affinity chromatography products for different applications.



GST-tagged Proteins

Glutathione S-transferase (GST) is a 26 kDa protein derived from *Schistosoma japonicum*. GST enzymes from various sources, both native and recombinantly expressed as fusion to the N-terminus of target proteins, are easily purified in a one-step procedure by affinity chromatography on immobilized glutathione (Glutathione Chromatrix™, Fast Flow).

Due to the positive influence of the GST-tag on protein solubility and expression efficiency especially of small proteins, this technique has been widely used to generate proteins for crystallization, molecular immunology studies and studies involving protein-protein and protein-DNA interactions.

However, the GST-tag might sometimes interfere with these downstream applications. In these cases it is easily removed by protease cleavage e.g. by Factor Xa (GST Cleavage Capture Kit) provided that a specific protease sequence is located between the protein domain and GST.

Furthermore, free GST or GST fusion proteins are conveniently detected by Western blotting using GST antibody.

Glutathione ChroMatrix™, Fast Flow

Suitable for FPLC, gravity flow and batch purification

6 % CL-Glutathione ChroMatrix™ is a superior, 6% cross-linked agarose support with covalently coupled glutathione attached via a 12-atom spacer. Its unique properties facilitate the rapid, high-yielding and economically priced one-step purification of some native Glutathione S-transferase (GST) enzymes and recombinant GST-tagged proteins. Furthermore, **6 % CL-Glutathione ChroMatrix™** is suitable for GST-pull down assays [1].

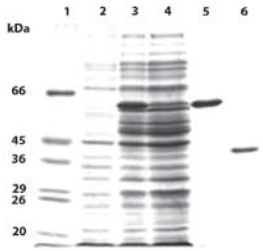
Features:

- Excellent binding capacity based on high surface area to volume ratio
- Fast flow rates
- Notable mechanical and chemical stability
- Reusable without any significant decrease in yield

Glutathione ChroMatrix™ characteristics	
Matrix	6 % cross-linked agarose
Linker	12-atom spacer
Bead size	70 – 145 µm
Binding capacity*	>20 mg / ml resin
Max. linear flow rate	600 cm/h
Maximum pressure	3 bar (43.5 psi)
pH stability	2-12
Chemical stability	Stable to all solutions commonly used in gel filtration including 8 M urea and 6 M guanidine hydrochloride **Stable in organic solvents!****

* tested with human Glutathione S-transferase (GST)
** Ethanol, DMF, THF, acetone, DMS, chloroform, dichlormethane, dichlorethane, pyridine, triethyl phosphate and acetonitrile

SDS-PAGE analysis of the purification of recombinant human biliverdin-IX reductase (BVR-A) [2]



lane 1: molecular mass markers (sizes indicated)
lane 2: lysate from cells transformed with control vector (pGEX-KG)
lane 3: lysate from cells transformed with pGEX-BVR-A (with IPTG)
lane 4: lysate from cells transformed with pGEX-BVR-A (without IPTG)
lane 5: purified GST-BVR-A
lane 6: cleaved BVR-A

Selected References:

[1] Bee *et al.* (2010) Growth and tumor suppressor NORE1A is a regulatory node between Ras signaling and microtubule nucleation. *J. Biol. Chem.* **285 (21)**:16258.

[2] Franklin *et al.* (2007) Activation of biliverdin-IXα reductase by inorganic phosphate and related anions. *Biochemical Journal* **405 (1)**:61.

6 % CL-Glutathione ChroMatrix™			
Product	**Cat.-No.**	**Amount**	**Price**
Bulk material	AC-201-10	10 ml	147,—
	AC-201-50	50 ml	682,50
	AC-201-100	100 ml	1.365,—
	AC-201-500	500 ml	5.250,—

GST Cleavage Capture Kit

The GST Cleavage Capture Kit allows easy release of free recombinant protein from their GST-tag when a Factor Xa recognition sequence is present.

Product	Cat.-No.	Amount	Price
GST Cleavage Capture Kit Removal of GST-tags by Factor Xa digestion of GST-fusion proteins	PR-946	1 Kit	140,—

GST Antibody

Affinity-purified rabbit polyclonal antibody raised against a recombinant full-length GST protein. It is suitable for the detection of GST and GST fusion proteins by Western Blot applications. α-GST reacts with the GST protein in HeLa nuclear extract by Western Blotting.

Selected references:

Ketterer (2001) A bird's eye view of the glutathione transferase field. *Chem. Biol. Interact.* **138**:27.
Smith *et al.* (1988) Single-step purification of polypeptides expressed in Escherichia colias fusion with glutathione-S-transferase. *Gene* **67**:31.
Zhan *et al.* (2001) Structural analysis of regulatory protein domains using GST-fusion proteins. *Gene* **281(1-2)**:1.
Toye *et al.* (1990) Immunologic Characterization of a Cloned Fragment Containing the Species-Specific Epitope from the Major Outer Membrane Protein of Chlamydia trachomatis. *Infect. Immun.* **58**:3909.
Fikrig *et al.* (1990) Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. *Science* **250**: 553.
Kaelin *et al.* (1991) Identification of cellular proteins that can interact specifically with the T/EIA-binding region of the retinoblastoma gene product. *Cell* **64**:521.
Kaelin *et al.* (1991) The T/E1A-binding domain of the retinoblastoma product can interact selectively with a sequence-specific DNA-binding protein. *Cell* **65**:1073.

Product	Cat.-No.	Amount	Price
α-GST anti-Glutathione S-transferase, rabbit, polyclonal	ABD-035	50 µg	400,—

His-tagged Proteins

Immobilized metal affinity chromatography (IMAC) is most frequently used for the purification of polyhistidine (His-) tagged proteins. This technique is based on the interaction between certain exposed protein residues (preferentially histidines) with transition metal cations (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}). The transition metal itself is immobilized to a cross-linked agarose matrix via a chelating group such as iminodiacetic acid (IDA) [1].

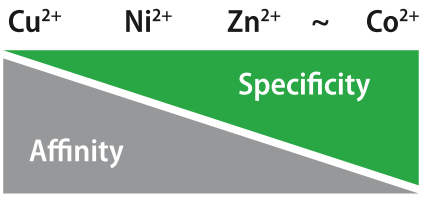
Successful purification experiments of His-tagged proteins strongly depend on the particular amino acid sequence, the protein conformation and the microenvironment and location of the His-tag (C- or N-terminal). Furthermore, undesired co-purification of non-specific host cell proteins is often observed, especially in the case of *E. coli* expression systems.

Alternative to common solution strategies e.g. the increase of salt or detergent concentration or the variation of the His-tag location optimization of the purification strategy can easily be achieved by

- variation of metal ion density since a lower amount of metal ions may minimize non-specific protein binding[2-4].
- changing the metal ion itself [2,3].

Based on the HSAP concept, IDA-immobilized Cu^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+} ions exhibit varying affinities & specificities towards the histidine tag [2,3].

e.g.: Co^{2+}-ions display a higher specificity with less binding affinity than Ni^{2+} ions towards histidines and are therefore an ideal option to reduce non-specific protein binding.



Selected References:

[1] Porath *et al.* (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature* **258**:598.

[2] Gaberc-Porekar *et al.* (2001) Perspectives of immobilized-metal affinity chromatography. *J. Biochem. Biophys. Methods* **49**:335.

[3] Ueda *et al.* (2003) Current and prospective applications of metal ion–protein binding. *Journal of Chromatography* **988**:1.

[4] Liesiene *et al.* (1997) Immobilized metal affinity chromatography of human growth hormone-effect of ligand density. *Journal of Chromatography* **764**:27.

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Nickel ChroMatrix™, Fast Flow

Suitable for FPLC, gravity flow and batch purification

6 % CL-Nickel ChroMatrix™ is a superior, 6 % cross-linked immobilized metal affinity chromatography (IMAC) resin that uses nickel ions for purifying recombinant polyhistidine (His-) tagged proteins. Its unique properties facilitate the rapid and high-yielding one-step purification from crude lysates both under denaturing and non-denaturing conditions.

Features:

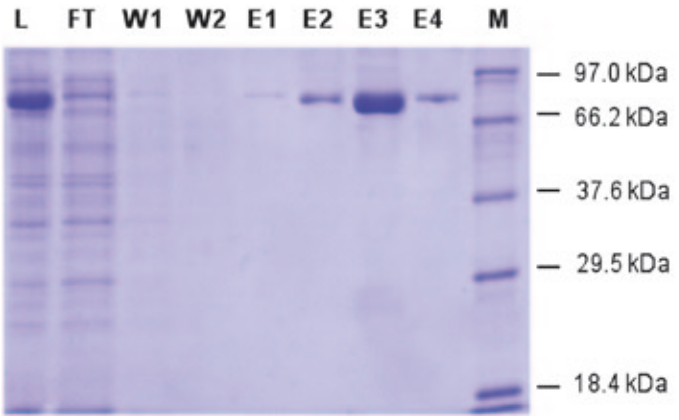
- Excellent binding capacity based on high surface area to volume ratio
- Fast flow rates
- Notable mechanical and chemical stability
- Reusable without any significant decrease in yield

Nickel ChroMatrix™ characteristics	
Matrix	6 % cross-linked agarose
Linker	Iminodiacetic acid (IDA)
Bead size	70–145 µm
Binding capacity*	> 20 mg / ml resin
Max. linear flow rate	600 cm/h
Maximum pressure	3 bar (43.5 psi)
pH stability	2–12
Chemical stability	Stable to all solutions commonly used in gel filtration including 8 M urea and 6 M guanidine hydrochloride

* tested with His-tagged human Glutathione S-transferase (GST)

SDS PAGE analysis of recombinant human ribonucleotide reductase (RNR) purification
His-tagged RNR was expressed in *E. coli* (30 liter culture), purified by one-step chromatography with 6 % CL-Nickel ChroMatrix™ (970 ml) and subsequently analysed by SDS-PAGE (equal amounts per lane).

Flow rate:
55 ml/min (column diameter: 5 cm, bed height: 60 cm)
Wash buffer:
50 mM H_2NaPO_4, 300 mM NaCl, 10 mM imidazole
Elution buffer:
50 mM H_2NaPO_4, 300 mM NaCl, 250 mM imidazole



L = Lysate, FT = Flow through, W1–W2 = Wash 1–2,
E1–E4 = Elution 1–4, M = Molecular weight marker

6 % CL-Nickel ChroMatrix™			
Product	**Cat.-No.**	**Amount**	**Price**
Bulk material	AC-202-10	10 ml	63,—
	AC-202-50	50 ml	252,—
	AC-202-100	100 ml	483,—
	AC-202-500	500 ml	2.362,50

Cobalt ChroMatrix™, Fast Flow

Suitable for FPLC, gravity flow and batch purification

6 % CL-Cobalt ChroMatrix™ is a superior, 6 % cross-linked immobilized metal affinity chromatography (IMAC) resin that uses cobalt ions for purifying recombinant polyhistidine (His-) tagged proteins. Its unique properties facilitate the rapid and high-yielding one-step purification from crude lysates both under denaturing and non-denaturing conditions.

Compared to Ni^{2+} ions, IDA-immobilized Co^{2+}-ions generally display a higher specificity towards histidines and are therefore an ideal option to reduce non-specific protein binding [1].

Features:

- Excellent binding capacity based on high surface area to volume ratio
- Fast flow rates
- Notable mechanical and chemical stability
- Reusable without any significant decrease in yield

Cobalt ChroMatrix™ characteristics	
Matrix	6 % cross-linked agarose
Linker	Iminodiacetic acid (IDA)
Bead size	70–145 µm
Binding capacity*	> 20 mg / ml resin
Max. linear flow rate	600 cm/h
Maximum pressure	3 bar (43.5 psi)
pH stability	2–12
Chemical stability	Stable to all solutions commonly used in gel filtration including 8 M urea and 6 M guanidine hydrochloride

* tested with His-tagged human Glutathione S-transferase (GST)

Selected References:

[1] Ueda *et al.* (2003) Current and prospective applications of metal ion–protein binding. *Journal of Chromatography* **988**:1.

6 % CL-Cobalt ChroMatrix™			
Product	**Cat.-No.**	**Amount**	**Price**
Bulk material	AC-203-10	10 ml	63,—
	AC-203-50	50 ml	252,—
	AC-203-100	100 ml	483,—
	AC-203-500	500 ml	2.362,50

Nickel, Zinc, Cobalt, Copper and Metal Free Agaroses, Gravity Flow

Suitable for gravity flow and batch purification

His-tagged proteins are efficiently purified by a one-step procedure from crude lysates both under denaturing and non-denaturing conditions.

Features:

- Tridentate IDA-linker for protein elution with lower imidazole concentrations compared to tetradentate chelators (e.g. NTA)
- Precharged material with four metal types (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}) as well as metal free agarose
- Two metal loading densities
- Different formats: bulk material, pre-packed columns and spin columns for standard microcentrifuge

Product characteristics	
Matrix	6 % cross-linked agarose
Bead size	50–150 µm
Linker	Iminodiacetic acid (IDA)
Metal loading capacity	**Low density:** 5–20 µmol Me^{2+}/ ml resin **High density:** 20–40 µmol Me^{2+}/ ml resin
Linear flow rate	26 cm/h
Maximum pressure	0.18 bar (2.6 psi)
Chemical stability	Stable to all solutions commonly used in gel filtration including 8 M urea and 6 M guanidine hydrochloride

Optimize your purification strategy by a flexible choice of metal ion and metal loading density

Increasing histidine binding specificity

Decreasing affinity

	Cu^{2+}	**Ni^{2+}**	**Zn^{2+}**	**Co^{2+}**
High density metal loading	n/a	High Density Nickel Agarose **(AC-303)**	High Density Zinc Agarose **(AC-305)**	High Density Cobalt Agarose **(AC-307)**
Low density metal loading	Low Density Copper Agarose **(AC-308)**	Low Density Nickel Agarose **(AC-304)**	Low Density Zinc Agarose **(AC-306)**	n/a

Based on the HSAP concept, **IDA-immobilized Cu^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+} ions exhibit different affinities & specificities towards histidines [2,3]. Copper (Cu^{2+}):** Shows the lowest specificity resulting in high target recoveris. Unspecific protein binding is minimized by low density metal loading. **Cobalt (Co^{2+})**: Shows the highest binding specificity resulting in reduced unspecific protein binding. Target loss is minimized by high density metal loading. **Nickel (Ni^{2+}) and Zinc (Zn^{2+})**: Show intermediate selectivity. While using Ni^{2+} ions is the standard method, IDA-immobilized Zn^{2+} may prove superior to either immobilized Cu^{2+} and Ni^{2+} ions, as a result of its relatively low binding affinity for *E. coli* host cell proteins [4]. **Low density metal loading** enhances the qualitative purification of recombinant protein but with lower target recoveries. **High density metal loading** results in greater purification of recombinant proteins. However, unwanted proteins within the sample will also be bound.

The most straightforward way to determine the best material for a particular purification task is the usage of one of our Chelate Kits

Selected References:

[1] Porath *et al.* (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature* **258**:598.

[2] Gaberc-Porekar *et al.* (2001) Perspectives of immobilized-metal affinity chromatography. *J. Biochem. Biophys. Methods* **49**:335.

[3] Ueda *et al.* (2003) Current and prospective applications of metal ion–protein binding. *Journal of Chromatography* **988**:1.

[4] Richard *et al.* (2000) Design of Affinity Tags for One-Step Protein Purification from Immobilized Zinc Columns. *Biotechnol. Prog.* **16**:86.

Bulk Resins, Spin Columns and Pre-packed Columns

High Density Nickel Affinity Material

Product	Cat.-No.	Amount	Price
Bulk material	AC-303-25	25 ml	145,—
	AC-303-50	50 ml	230,—
	AC-303-100	100 ml	400,—
	AC-303-500	500 ml	1.800,—
His-Spin Column (100 µl each)	AC-303SPC-25	25 columns	160,—
	AC-303SPC-50	50 columns	290,—
His-Column (1 ml each)	AC-303C	8 columns	150,—
HisXL-Column (5 ml each)	AC-303C-5	5 columns	410,—

Low Density Nickel Affinity Material

Product	Cat.-No.	Amount	Price
Bulk material	AC-304-25	25 ml	145,—
	AC-304-50	50 ml	230,—
	AC-304-100	100 ml	400,—
	AC-304-500	500 ml	1.800,—
His-Spin Column (100 µl each)	AC-304SPC-25	25 columns	160,—
	AC-304SPC-50	50 columns	290,—
His-Column (1 ml each)	AC-304C	8 columns	150,—
HisXL-Column (5 ml each)	AC-304C-5	5 columns	410,—

High Density Zinc Affinity Material

Product	Cat.-No.	Amount	Price
Bulk material	AC-305-25	25 ml	145,—
	AC-305-50	50 ml	230,—
	AC-305-100	100 ml	400,—
	AC-305-500	500 ml	1.800,—
His-Spin Column (100 µl each)	AC-305SPC-25	25 columns	160,—
	AC-305SPC-50	50 columns	290,—
His-Column (1 ml each)	AC-305C	8 columns	150,—
HisXL-Column (5 ml each)	AC-305C-5	5 columns	410,—

Low Density Zinc Affinity Material

Product	Cat.-No.	Amount	Price
Bulk material	AC-306-25	25 ml	145,—
	AC-306-50	50 ml	230,—
	AC-306-100	100 ml	400,—
	AC-306-500	500 ml	1.800,—
His-Spin Column (100 µl each)	AC-306SPC-25	25 columns	160,—
	AC-306SPC-50	50 columns	290,—
His-Column (1 ml each)	AC-306C	8 columns	150,—
HisXL-Column (5 ml each)	AC-306C-5	5 columns	410,—

High Density Cobalt Affinity Material

Product	Cat.-No.	Amount	Price
Bulk material	AC-307-25	25 ml	145,—
	AC-307-50	50 ml	230,—
	AC-307-100	100 ml	400,—
	AC-307-500	500 ml	1.800,—
His-Spin Column (100 µl each)	AC-307SPC-25	25 columns	160,—
	AC-307SPC-50	50 columns	290,—
His-Column (1 ml each)	AC-307C	8 columns	150,—
HisXL-Column (5 ml each)	AC-307C-5	5 columns	410,—

Low Density Copper Affinity Material

Product	Cat.-No.	Amount	Price
Bulk material	AC-308-25	25 ml	145,—
	AC-308-50	50 ml	230,—
	AC-308-100	100 ml	400,—
	AC-308-500	500 ml	1.800,—
His-Spin Column (100 µl each)	AC-308SPC-25	25 columns	160,—
	AC-308SPC-50	50 columns	290,—
His-Column (1 ml each)	AC-308C	8 columns	150,—
HisXL-Column (5 ml each)	AC-308C-5	5 columns	410,—

High Density Metal Free Affinity Material

Product	Cat.-No.	Amount	Price
Bulk material	AC-301-25	25 ml	145,—
	AC-301-50	50 ml	230,—
	AC-301-100	100 ml	400,—
	AC-301-500	500 ml	1.800,—
Spin Column (100 µl each)	AC-301SPC-25	25 columns	160,—
	AC-301SPC-50	50 columns	290,—
Column (1 ml each)	AC-301C	8 columns	150,—
XL-Column (5 ml each)	AC-301C-5	5 columns	410,—

Low Density Metal Free Affinity Material

Product	Cat.-No.	Amount	Price
Bulk material	AC-302-25	25 ml	145,—
	AC-302-50	50 ml	230,—
	AC-302-100	100 ml	400,—
	AC-302-500	500 ml	1.800,—
Spin Column (100 µl each)	AC-302SPC-25	25 columns	160,—
	AC-302SPC-50	50 columns	290,—
Column (1 ml each)	AC-302C	8 columns	150,—
XL-Column (5 ml each)	AC-302C-5	5 columns	410,—

Test Kits

Product	Application	Cat.-No.	Price
Nickel Chelate Kit Includes: • 2 ml High Density Nickel • 2 ml Low Density Nickel	Recommended for proteins that are easy to separate	AC-401	40,—
Nickel Chelate Kit **+ 20 empty mini columns**	Recommended for proteins that are easy to separate	AC-401L	65,—
Nickel & Cobalt Chelate Kit Includes: • 2 ml High Density Nickel • 2 ml Low Density Nickel • 2 ml High Density Cobalt	Recommended for proteins that are easy to separate	AC-402	60,—
Nickel & Cobalt Chelate Kit **+ 30 empty mini columns**	Recommended for proteins that are easy to separate	AC-402L	95,—
Zinc Chelate Kit Includes: • 2 ml High Density Zinc • 2 ml Low Density Zinc	Recommended for proteins that are difficult to separate	AC-403	40,—
Zinc Chelate Kit **+ 20 empty mini columns**	Recommended for proteins that are difficult to separate	AC-403L	65,—
Zinc & Copper Chelate Kit Includes: • 2 ml High Density Zinc • 2 ml Low Density Zinc • 2 ml Low Density Copper	Recommended for proteins that are difficult to separate	AC-404	60,—
Zinc & Copper Chelate Kit **+ 30 empty mini columns**	Recommended for proteins that are difficult to separate	AC-404L	95,—
High Density Chelate Kit Includes: • 2 ml High Density Metal Free • 2 ml High Density Nickel • 2 ml High Density Zinc • 2 ml High Density Cobalt	High Binding Capacity	AC-405	70,—
High Density Chelate Kit **+ 40 empty mini columns**	High Binding Capacity	AC-405L	115,—
Low Density Chelate Kit Includes: • 2 ml Low Density Metal Free • 2 ml Low Density Nickel • 2 ml Low Density Zinc • 2 ml Low Density Copper	High Selectivity	AC-406	70,—
Low Density Chelate Kit **+ 40 empty mini columns**	High Selectivity	AC-406L	115,—

Antibody Immobilization

Immobilized or purified antibodies, also termed immunoglobulins, are of major importance both for immunochemical techniques in basic research (e.g. Immunoprecipitation) and for diagnostic applications.

One of the most successful immobilization and purification technique is based on the high affinity and binding specificity of Protein A, a surface protein of *Staphylococcus aureus*, towards the Fc region of a broad range of mammalian immunoglobulins (Ig).

Immunoglobulins from different species and different isotypes within a species (IgA, IgG, IgM, IgE, IgD) differ in their affinity to Protein A [1,2].

Refer to the tables below for a comprehensive overview of the relative affinity of immobilized Protein A for various species and subclasses of polyclonal and monoclonal IgGs [1].

Relative avidity of Protein A for polyclonal antibodies

Species / Subclass (Polyclonal)	Protein A
Rabbit	++++
Cow	++
Horse	++
Goat	-
Guinea pig	++++
Sheep	+/-
Pig	+++
Rat	+/-
Mouse	++
Chicken	-
Human IgG	++++
Human IgM	-
Human IgD	-
Human IgA	-

++++ (strong binding) – (weak or no binding)

Relative affinity of Protein A for various isotypes

Species / Subclass (Monoclonal)	Protein A
Human	
IgG_1	++++
IgG_2	++++
IgG_3	-
IgG_4	++++
Mouse	
IgG_1	+
IgG_{2a}	++++
IgG_{2b}	+++
IgG_3	++
Rat	
IgG_1	-
IgG_{2a}	-
IgG_{2b}	-
IgG_{2c}	+

Protein A Agarose

Protein A is a cell wall component of *Staphylococcus aureus*. It consists of a single polypeptide chain shaped as a cylinder, which contains five antibody binding domains. These high affinity regions are specifically bound to the Fc region of immunoglobulins (IgGs).

Protein A is temperature stable and it retains its native conformation even in the presence of denaturing agents. Protein A resins have been widely used to purify a broad range of immunoglobulins from different mammalian species.

Features:

- High IgG-binding capacity
- Milder elution condition than Protein G resin
- High stability binding of Protein A: resin is reusable with no significant loss of binding capacity

Protein A Agarose characteristics	
Matrix	4 % cross-linked agarose
Bead size	40–180 µm
Binding capacity	~ 25 mg human IgG / ml resin
Ligand density	~ 3 mg rProtein A / ml resin
Linear flow rate	26 cm/h
Maximum pressure	0.18 bar (2.6 psi)

Selected References:

[1] Harlow and Lane (eds.) (1988) *Antibodies. A Laboratory Manual.* Cold Spring Harbor Laboratory, N.Y., **617–618.**

[2] Richmann *et al.* (1982) The binding of staphylococcal protein A by the sera of different animal species. *J. Immunol.* **128**:2300.

Protein A Agarose			
Product	**Cat.-No.**	**Amount**	**Price**
Bulk material	AC-309-5	5 ml	360,—
	AC-309-10	10 ml	640,—
	AC-309-25	25 ml	1.250,—
	AC-309-50	50 ml	2.450,—
Protein A Agarose Column (1 ml per column)	AC-309C-1	1 Column	200,—
Protein A Agarose XL Column (5 ml per column)	AC-309-5	1 Column	500,—

Nucleotide binding Proteins

Immobilized nucleotides provide a convenient and rapid purification procedure for a large number of proteins such as kinases, GTPases, chaperones, motor proteins and others.

Jena Bioscience offers affinity chromatography material that is taylor-made for purification (or for related assays) of particular (d)NTP-binding proteins since we produce NTPs and dNTPs that are linked to the matrix at various positions of sugar, base or phosphate moiety of the nucleotide, offer different types and lengths of linkers and provide different types of chromatography material ranging from bulk media to pre-packed columns that fit any machine.

Immobilized Adenosine Nucleotides:	**ATP** Page 16	**dATP** Page 18	**ADP** Page 18	**AMP** Page 18	**cAMP** Page 19
	Affinity Chromatography Kits for ATP binding Proteins Page 20				
Immobilized Guanosine and m^{7}Guanosine Nucleotides:	**GTP** Page 22	**dGTP** Page 22	**m^{7}GTP** Page 23	**m^{7}GDP** Page 23	**cGMP** Page 24
Immobilized Cytidine Nucleotides:	**CTP** Page 24	**dCTP** Page 25			
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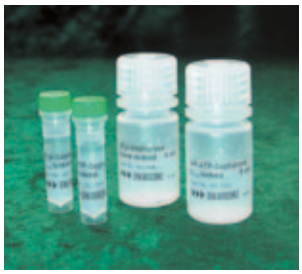
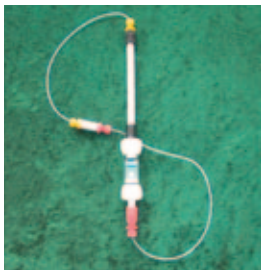
Immobilized Xanthosine Nucleotides:	**XTP** Page 28				

Different types of chromatography material available

All our affinity chromatography materials carrying nucleotides or amino/phospho-amino acids are available as bulk media, pre-packed MPLC columns, syringe columns and screening columns. Please refer to the product pages to access the product lists and data sheets, and to order affinity chromatography products.





Bulk material	Pre-packed syringe column	Pre-packed screening column	Pre-packed MPLC column
based on Agarose (equals Sepharose™ 4B)	based on Agarose (equals Sepharose™ 4B)	based on Agarose (equals Sepharose™ 4B) ▪ maximum amount 0.6 ml ▪ ready-to-use glass columns ▪ one each upper and lower frit ▪ upper and lower end piece ▪ special 96-well-MTP format column holder on request	based on Toyopearl® AF-650M ▪ 40 – 90 µ material ▪ 1× Omnifit column kit 10 × 50 mm ▪ 1× adjustable end piece ▪ 2× female-female (1/4" to 6 mm) coupling ▪ 2 m PTFE tubing (1/16" OD × 0.8 mm ID) MPLC columns are delivered with adapters for FPLC Systems of BioRad, GE Healthcare (Äkta™) and Pharmacia.

Toyopearl® is a trademark of TOSOH Bioscience
Äkta™ and Sepharose™ are trademarks of GE Healthcare Companies

Properties of Agarose material

Agarose content	4 %
Bead size	45–165 µm
p_{max}	0.25 bar
Degree of substitution	See product data sheets
pH stability (short term)	4–9
pH stability (long term)	7.5
Recommended linear fllow rate	11.5 cm/h
Chemical stability	Stable to all solutions commonly used in gel filtration, including 8 M urea and 6 M guanidine hydrochloride. **Not stable in organic solvents!**

Properties of Toyopearl AF-650M material

Particle size distribution (>80 % within range)	40–90 µm
Mean pore diameter	1000 Å
p_{max}	3 bar
Degree of substitution	See product data sheets
pH stability (short term)	4–9
pH stability (long term)	7.5
Recommended linear fllow rate	60–600 cm/h
Resin volume	1 g equals ca. 3.5 ml settled swollen resin volume
Chemical stability	Stable to all solutions commonly used in gel filtration, including 8 M urea and 6 M guanidine hydrochloride. **Not stable in organic solvents!**

General remarks for Affinity Materials carrying immobilized Nucleotides

Binding capacity of the column:

It is impossible to predict a precise capacity of the material, because this very much depends on the protein itself and on other compounds contained in the mixture to be separated. The degree of substitution which allows a very rough estimate of binding capacity is given in the product data sheets.

Handling of Agarose and Toyopearl® AF-650M affinity materials:

For stability of the nucleotide moiety, the material must be kept at low temperature (4 °C) and within a pH range of 6.0 to 8.5 during all operations. Long-term storage at pH 7.5 at 4 °C with 20 % Ethanol.

Toyopearl® AF-650M may be used at higher pressure (p_{max}=7 bar) compared to Agarose (p_{max}=0.25 bar).

Regeneration of the affinity material:

Since the specific effects of other components or impurities in crude mixtures onto the material can not be predicted we specify the material for single use only. We do not recommend using affinity resins multiple times.

Please note: If your sample contains enzymes (even traces) that are able to degrade nucleotides (such as phosphatases), the material may show less or no affinity for nucleotide binding proteins. For example, ATP can be degraded to ADP by phosphatases and ADP shows no or a largely reduced affinity to many ATP binding proteins. This is an issue particularly with material in which the nucleotide is bound via a different position than the γ-phosphate. However, even γ-linked materials can be degraded under certain circumstances.

Nevertheless, if you decide to regenerate the affinity matrix, please read the notes below:

General protocol:

- It is recommended to use a buffer with an ionic strength of 0.15 M or greater to prevent unspecific ionic interactions between the solute molecules and the matrix. A flow rate of 15 cm/h is recommended.
- To avoid clogging of column filters, it is recommended to filter or centrifuge the sample before applying it to the column to get rid of precipitated material.
- Before applying the sample, equilibrate the column with at least two column volumes of the sample buffer.
- Sample application: It is recommended to use at least a 2fold excess of the Agarose (by degree of substitution, see above) over the protein to be purified.
- Washing: Wash the columns with at least 2–3 column volumes of a suitable washing buffer.
- Elution: Use 0.1 M solution of the nucleotide in a suitable buffer to elute the nucleotide binding protein from the matrix.

Regeneration (make sure you read the notes above):

The material may be attempted for re-use by regenerating with at least 2–3 column volumes of alternating high pH (0.1 M Tris-HCl + 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate + 0.5 M NaCl, pH 6.0) buffers. This procedure should be repeated 3 times followed by re-equilibration.

In some applications, substances such as denatured proteins or lipids do not elute in the regeneration procedure. These can be removed by washing the column with 2–3 column volumes of a non-ionic detergent solution, e.g. 0.1 % Triton X-100 followed by at least 2–3 column volumes of distilled water. Wash the column with 2–3 column volumes of storage buffer before storage, or use the same amount of sample buffer if you want to start a new purification run.

MPLC columns are delivered with adapters for FPLC Systems of BioRad, GE Healthcare (Äkta™) and Pharmacia.

Immobilized Adenosine Nucleotides

Adenosine triphosphate (ATP)-affinity chromatography has been widely used to purify various ATP binding proteins such as kinases [1-4], β-and γ-glutamate decarboxylase [5] and chaperones [6–9].

Efficient protein binding strongly depends on and the type of ATP-matrix attachment [4,10–11].

Jena Bioscience provides a large selection of ready-to-use ATP affinity chromatography material:

- ATP immobilized at different positions:
  Adenine base moiety: C-8, N-6
  Ribose moiety: 2'/3'-OH
  Phosphate moiety: γ-Phosphate
- several types and lengths of linkers
- different types of chromatography material ranging from bulk material to pre-made columns

Usage tips for affinity materials carrying immobilized ATP:

1. ATP-Agaroses will bind ATP binding proteins. The original reference characterizing these products is [4].
2. AP-ATP-Agarose (AC-101) is immobilized via a phenyl moiety. This mimics a tyrosine residue, causing the material to be most suitable for tyrosine kinases (although serine/threonine kinases will also bind).
3. Blocking of the ATP-Agarose is important when purifying proteins from crude fractions where protein concentration is low, as this prevents non-specific binding to the Agarose matrix. A blocking method is described in the reference above. (For instance, pass crude cell extract over a column, and then regenerate the matrix with 10 mM ATP and high salt (0.5 M NaCl), followed by re-equilibration with several bead volumes of binding buffer).
4. Any protein with an accessible ATP binding domain will bind to any ATP-Agarose, regardless of activation state however, activated proteins will bind with a higher affinity than the corresponding inactivated protein.
5. Proteins in which the ATP binding domain is "hidden" in the inactive state require activation to reveal the ATP binding domain and enable binding to the Agarose.
6. Dehydrogenases can be purified using ATP-Agarose by omitting the NADH from the binding buffer, and using 10 mM NADH as the specific elutant.
7. Heat Shock Proteins (HSP) can be purified using ATP-Agarose by omitting ADP from the binding buffer and using 10 mM ADP as the specific elutant.
8. Better recovery and yields may be obtained when the ATP-Agarose purification is followed by ion-exchange chromatography. This is reverse of how most researchers use the ATP-Agarose for purification.
9. β-Glycerol Phosphate is not recommended as a buffer component for the purification of kinases. Some users have noted that using this component has resulted in reduced activity and stability of some protein kinases.

Selected references:

[1] Jeansonne *et al.* (2006) A rapid ATP affinity-based purification for the human non-receptor tyrosine kinase c-Src. *Protein Expression and Purification* **46**:240.

[2] Dhillon *et al.* (2009) The C-terminus of Raf-1 acts as a 14-3-3-dependent activation switch. *Cellular Signalling* **21 (11)**:1645.

[3] Mlakar *et al.* (2006) Citrate Inhibition-Resistant Form of 6-Phosphofructo-1-Kinase from *Aspergillus niger.* *Applied and Environmental Microbiology* **72 (7)**:4515.

[3] Ramadoss *et al.* (1976) Affinity chromatography of phosphofructokinase. *Arch. Biochem. Biophys.* **175 (2)**:487.

[4] Haystead *et al.* (1983) Gamma-phosphate-linked ATP-sepharose for the affinity purification of protein kinases- rapid purification to homogeneity of skeletal muscle mitogen-activated protein kinase kinase. *Eur. J. Biochem.* **214 (2)**:459.

[5] Wu *et al.* (1984) Binding of ATP to brain glutamate decarboxylase as studied by affinity chromatography. J. Neurochem. **42 (6)**:1607.

[6] Bendz *et al.* (2007) Human heat shock protein 70 enhances tumor antigen presentation through complex formation and intracellular antigen delivery without innate immune signaling. J. Biol. Chem. **282 (43)**:31688.

[7] Place *et al.* (2001) Temperature interactions of the molecular chaperone Hsc70 from the eurythermal marine goby *Gillichthys Mirabilis.* *Journal of Experimental Biology* **204**:2675.

[8] Kumaraguru *et al.* (2000) Involvement of an ATP-Dependent Peptide Chaperone in Cross-Presentation after DNA Immunization. *Journal of Immunology* **165**:750.

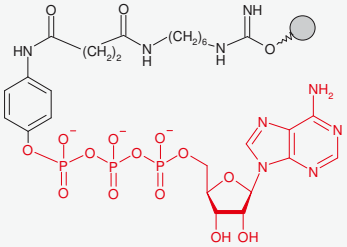
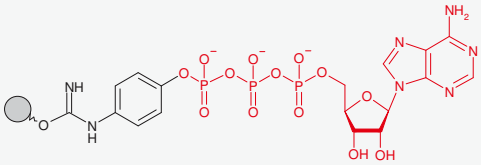
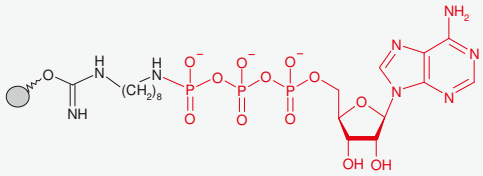
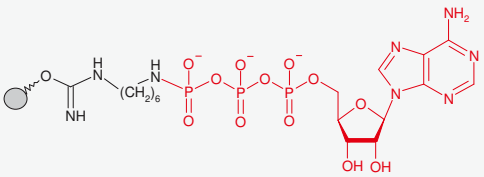
[9] Welch *et al.* (1985) Rapid Purification of Mammalian 70,000-Dalton Stress Proteins: Affinity of the Proteins for Nucleotides. *Molecular and Cellular Biology* **5 (6)**:1229.

[10] Jeno *et al.* (1989) Purification and Charaterization of a 40 S Ribosomal Protein S6 Kinase from Vanadate-stimulated Swiss 3T3 Cells. *J. Biol. Chem.* **264**:1293.

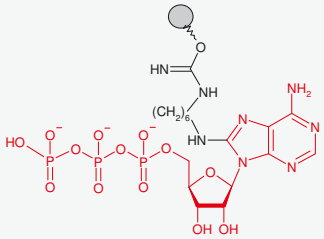
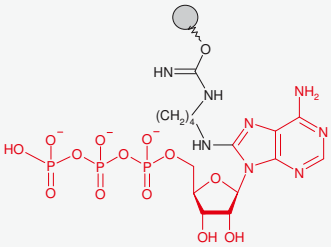
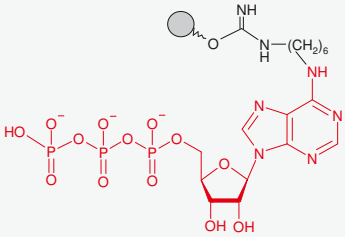
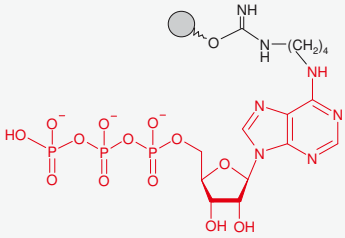
[11] Trayer *et al.* (1974) Affinity Chromatography of Nicotinamide Nucleotide-Dependent Dehydrogenases on Immobilized Nucleotide Derivates. *Biochem. J.* **139**:609.

ATP

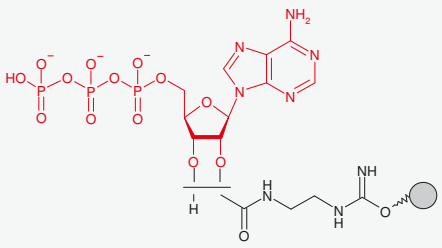
Linked via the γ-Phosphate

Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
γ-Aminophenyl-ATP AC-101	γ-Phosphate 10-atom carbon spacer 	AC-101S	Bulk material 1 ml	147,—
		AC-101L	Bulk material 5 ml	588,—
		AC-101C	Pre-packed syringe column 1 ml	168,—
		AC-101SC	Pre-packed screening column 0.2 ml	45,15
		AC-101MP	Pre-packed MPLC column 1 ml	672,—
γ-Aminophenyl-ATP AC-102	γ-Phosphate without spacer 	AC-102S	Bulk material 1 ml	115,50
		AC-102L	Bulk material 5 ml	462,—
		AC-102C	Pre-packed syringe column 1 ml	136,50
		AC-102SC	Pre-packed screening column 0.2 ml	38,85
		AC-102MP	Pre-packed MPLC column 1 ml	640,50
γ-Amino-octyl-ATP AC-105	γ-Phosphate 8-atom carbon spacer 	AC-105S	Bulk material 1 ml	140,—
		AC-105L	Bulk material 5 ml	560,—
		AC-105C	Pre-packed syringe column 1 ml	160,—
		AC-105SC	Pre-packed screening column 0.2 ml	43,—
		AC-105MP	Pre-packed MPLC column 1 ml	640,—
γ-Amino-hexyl-ATP AC-116	γ-Phosphate 6-atom carbon spacer 	AC-116S	Bulk material 1 ml	140,—
		AC-116L	Bulk material 5 ml	560,—
		AC-116C	Pre-packed syringe column 1 ml	160,—
		AC-116SC	Pre-packed screening column 0.2 ml	43,—
		AC-116MP	Pre-packed MPLC column 1 ml	640,—

Linked via the Nucleobase

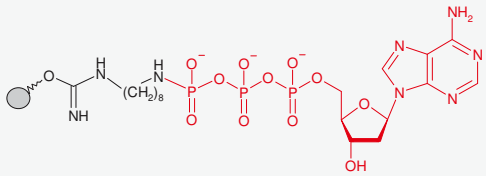
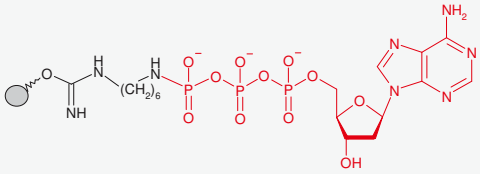
Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
8-Amino-hexyl-ATP* AC-127	C-8 6-atom carbon spacer 	AC-127S	Bulk material 1 ml	55,—
		AC-127L	Bulk material 5 ml	220,—
		AC-127C	Pre-packed syringe column 1 ml	75,—
		AC-127SC	Pre-packed screening column 0.2 ml	26,—
		AC-127MP	Pre-packed MPLC column 1 ml	550,—
8-Amino-butyl-ATP* AC-128	C-8 4-atom carbon spacer 	AC-128S	Bulk material 1 ml	55,—
		AC-128L	Bulk material 5 ml	220,—
		AC-128C	Pre-packed syringe column 1 ml	75,—
		AC-128SC	Pre-packed screening column 0.2 ml	26,—
N^6-(6-Amino)hexyl-ATP* AC-129	N-6 6-atom carbon spacer 	AC-129S	Bulk material 1 ml	55,—
		AC-129L	Bulk material 5 ml	220,—
		AC-129C	Pre-packed syringe column 1 ml	75,—
		AC-129SC	Pre-packed screening column 0.2 ml	26,—
		AC-129MP	Pre-packed MPLC column 1 ml	550,—
N^6-(4-Amino)butyl-ATP* AC-130	N-6 4-atom carbon spacer 	AC-130S	Bulk material 1 ml	55,—
		AC-130L	Bulk material 5 ml	220,—
		AC-130C	Pre-packed syringe column 1 ml	75,—
		AC-130SC	Pre-packed screening column 0.2 ml	26,—
		AC-130MP	Pre-packed MPLC column 1 ml	550,—

Linked via the Ribose

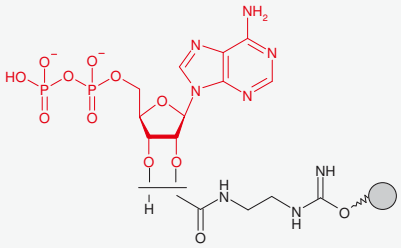
Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
2'/3'-EDA-ATP* AC-131	2'/3'-OH EDA 	AC-131S	Bulk material 1 ml	231,—
		AC-131L	Bulk material 5 ml	924,—
		AC-131C	Pre-packed syringe column 1 ml	252,—
		AC-131SC	Pre-packed screening column 0.2 ml	61,95
		AC-131MP	Pre-packed MPLC column 1 ml	756,—

* Phosphorous chain may be degraded by NTP/dNTP hydrolyzing enzymes.

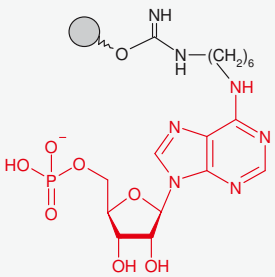
dATP

Linked via the γ-Phosphate				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
γ-Amino-octyl-dATP AC-111	γ-Phosphate 8-atom carbon spacer 	AC-111S	Bulk material 1 ml	189,—
		AC-111L	Bulk material 5 ml	756,—
		AC-111C	Pre-packed syringe column 1 ml	210,—
		AC-111SC	Pre-packed screening column 0.2 ml	53,55
		AC-111MP	Pre-packed MPLC column 1 ml	714,—
γ-Amino-hexyl-dATP AC-122	γ-Phosphate 6-atom carbon spacer 	AC-122S	Bulk material 1 ml	180,—
		AC-122L	Bulk material 5 ml	720,—
		AC-122C	Pre-packed syringe column 1 ml	200,—
		AC-122SC	Pre-packed screening column 0.2 ml	51,—
		AC-122MP	Pre-packed MPLC column 1 ml	680,—

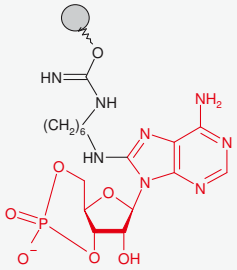
ADP

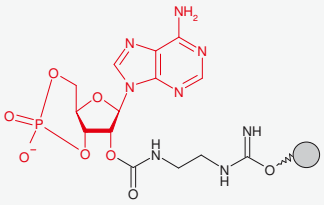
Linked via the Ribose				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
2'/3'-EDA-ADP AC-144	2'/3'-OH EDA 	AC-144S	Bulk material 1 ml	50,—
		AC-144L	Bulk material 5 ml	200,—
		AC-144C	Pre-packed syringe column 1 ml	80,—
		AC-144SC	Pre-packed screening column 0.2 ml	25,—
		AC-144MP	Pre-packed MPLC column 1 ml	550,—

AMP

Linked via the Nucleobase				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
N^6-(6-Amino)hexyl-AMP AC-145	N-6 6-atom carbon spacer 	AC-145S	Bulk material 1 ml	50,—
		AC-145L	Bulk material 5 ml	200,—
		AC-145C	Pre-packed syringe column 1 ml	80,—
		AC-145SC	Pre-packed screening column 0.2 ml	25,—
		AC-145MP	Pre-packed MPLC column 1 ml	550,—

cAMP

Linked via the Nucleobase				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
8-Amino-hexyl-cAMP AC-146	C-8 6-atom carbon spacer 	AC-146S	Bulk material 1 ml	180,—
		AC-146L	Bulk material 5 ml	720,—
		AC-146C	Pre-packed syringe column 1 ml	200,—
		AC-146SC	Pre-packed screening column 0.2 ml	51,—
		AC-146MP	Pre-packed MPLC column 1 ml	680,—

Linked via the Ribose				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
2'-EDA-cAMP AC-147	2'-OH EDA 	AC-147S	Bulk material 1 ml	180,—
		AC-147L	Bulk material 5 ml	720,—
		AC-147C	Pre-packed syringe column 1 ml	200,—
		AC-147SC	Pre-packed screening column 0.2 ml	51,—
		AC-147MP	Pre-packed MPLC column 1 ml	680,—

Affinity Chromatography Kits for ATP binding Proteins

ATP Affinity Test Kit

The **ATP Affinity Test Kit** provides a quick and easy way to determine the most suitable ATP-Agarose derivative for the purification of ATP binding proteins.

The kit contains everything to screen a crude protein solution for the most suitable chromatographic material.

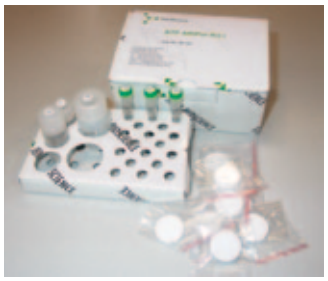
ATP affinity chromatography is used for the purification of a very large number of proteins, such as kinases, motor proteins and chaperones. There is however, a fundamental problem with using ATP in affinity chromatography: for attachment to a matrix ATP needs to be chemically modified with a linker. This linker may interfere with the protein-ATP interaction and thereby reduce the binding.

This problem can usually be circumvented by attaching ATP at a different position at the adenine base, the ribose, or the phosphate moiety. The **ATP Affinity Test Kit** contains a set of 4 typical ATP-Agarose chromatography materials, two with ATP linked via the adenine base, one via the ribose and one via the phosphate residue.

For purification of ATP binding proteins in preparative scale, our **ATP AffiPur Kits** should be used.

Product	Cat.-No.	Amount	Price
ATP Affinity Test Kit Screening Kit for the purification of ATP binding proteins	AK-102	1 Kit	189,—

ATP AffiPur Kits



The **ATP AffiPur Kits** are designed for the fast and easy purification of ATP binding proteins. Each kit contains one affinity chromatography material carrying immoblized ATP plus all the buffers and components required for the purification procedure - just follow the protocol from the user's guide.

Binding capacity of an ATP binding protein strongly depends on how the ATP is attached to the matrix. It is therefore recommended to determine the most suitable ATP affinity material before starting to purify a new protein using the **ATP Affinity Test Kit**.

All components of the kits are also available separately. Please see the Affinity Kit Components section for details.

ATP AffiPur Kit I

For the purification of ATP binding proteins using γ-Aminophenyl-ATP-Agarose

Product	Cat.-No.	Amount	Price
S Kit	AK-103S	1 Kit	180,—
L Kit	AK-103L	1 Kit	630,—

Kit Contents:

- Aminophenyl-ATP-Agarose, C_{10}-spacer
- PBS Tablets
- 200 mM Sodium Orthovanadate, activated
- 100× Protease Inhibitor Mix
- 5× Binding Buffer
- 5× Wash Buffer
- 5× Elution Buffer

ATP AffiPur Kit II

For the purification of ATP binding proteins using 8-[(6-Amino)hexyl]-amino-ATP-Agarose

Product	Cat.-No.	Amount	Price
S Kit	AK-104S	1 Kit	120,—
L Kit	AK-104L	1 Kit	420,—

Kit Contents:

- 8-[(6-Amino)hexyl]-amino-ATP-Agarose
- PBS Tablets
- 200 mM Sodium Orthovanadate, activated
- 100× Protease Inhibitor Mix
- 5× Binding Buffer
- 5× Wash Buffer
- 5× Elution Buffer

ATP AffiPur Kit III

For the purification of ATP binding proteins using N^6-(6-Amino)hexyl-ATP-Agarose

Product	Cat.-No.	Amount	Price
S Kit	AK-105S	1 Kit	120,—
L Kit	AK-105L	1 Kit	420,—

Kit Contents:

- N^6-(6-Amino)hexyl-ATP-Agarose
- PBS Tablets
- 200 mM Sodium Orthovanadate, activated
- 100× Protease Inhibitor Mix
- 5× Binding Buffer
- 5× Wash Buffer
- 5× Elution Buffer

ATP AffiPur Kit IV

For the purification of ATP binding proteins using 2'/3'-EDA-ATP-Agarose

Product	Cat.-No.	Amount	Price
S Kit	AK-106S	1 Kit	280,—
L Kit	AK-106L	1 Kit	980,—

Kit Contents:

- 2'/3'-EDA-ATP-Agarose
- PBS Tablets
- 200 mM Sodium Orthovanadate, activated
- 100× Protease Inhibitor Mix
- 5× Binding Buffer
- 5× Wash Buffer
- 5× Elution Buffer

Affinity Kit Components

Components of the **ATP AffiPur Kits** and the **ATP Affinity Test Kit** for use with our affinity chromatography materials carrying immoblized ATP. For more information about affinity purification of ATP binding proteins, please consult the user's guides of the **ATP AffiPur Kits.**

ATP Binding Buffer (5×)

For the purification of ATP binding proteins

Product	Cat.-No.	Amount	Price
S pack	AK-102B-S	15 ml	20,—
L pack	AK-102B-L	75 ml	80,—

Contains: Hepes, NaCl, $MgCl_2$, and NP-40

5× Binding Buffer for the use in the purification procedure of ATP binding proteins

Dilute 1:5 in ddH_2O

Store at 4°C
Stable for 1 year

ATP Wash Buffer (5×)

For the purification of ATP binding proteins

Product	Cat.-No.	Amount	Price
S pack	AK-102W-S	10 ml	20,—
L pack	AK-102W-L	50 ml	80,—

Contains: Hepes, NaCl, $MgCl_2$, and NP-40

5× Wash Buffer for the use in the purification procedure of ATP binding proteins

Dilute 1:5 in ddH_2O

Store at 4°C
Stable for 1 year

ATP Elution Buffer (5×)

For the purification of ATP binding proteins

Product	Cat.-No.	Amount	Price
S pack	AK-102E-S	2 ml	20,—
L pack	AK-102E-L	10 ml	80,—

Contains: Hepes, ATP, and NP-40

5× Elution Buffer for the use in the purification procedure of ATP binding proteins

Dilute 1:5 in ddH_2O

Store at -20°C
Stable for 1 year

Protease Inhibitor Mix (100×)

For the purification of proteins

Product	Cat.-No.	Amount	Price
S pack	AK-102I-S	0.5 ml	20,—
L pack	AK-102I-L	2.5 ml	80,—

The 100× Protease Inhibitor Mix can be used in the purification procedure of ATP binding proteins or for general protein purification procedures.

Contains: PMSF, Pefabloc, Aprotinin and Pepstatin A in methanol

5× Elution Buffer for the use in the purification procedure of ATP binding proteins

Dilute 1:100 in purification buffers

Store at -20°C
Stable for 1 year

Sodium Orthovanadate (1000×)

ATPase Inhibitor

Product	Cat.-No.	Amount	Price
S pack	AK-102V-S	100 µl	20,—
L pack	AK-102V-L	500 µl	80,—

Sodium Orthovanadate is an inhibitor of ATPase, alkaline phosphatase and tyrosine phosphatase that can be used in the purification procedure of ATP binding proteins.

Activated, pH 10.0

Dilute 1:1000 in purification buffers

Store at -20°C
Stable for 1 year

PBS Tablets

Phosphate Buffered Saline, Tablets

Product	Cat.-No.	Amount	Price
S pack	AK-102P-S	10 tablets	20,—
L pack	AK-102P-L	50 tablets	80,—

Phosphate Buffered Saline – for basic applications in Molecular and Cell Biology.

Can be used for instance in the purification procedure of ATP binding proteins.

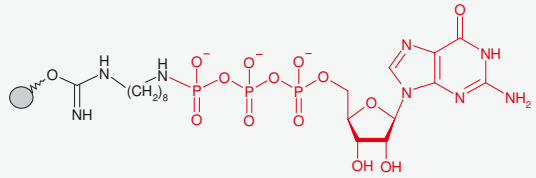
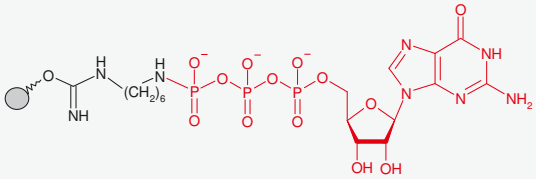
Each 5 g tablet should be dissolved in 500 ml ddH_2O to get 1× PBS (140 mM NaCl, 10 mM Phosphate Buffer, 3 mM KCl, pH 7.45).

Store at room temperature
Stable for 2 years

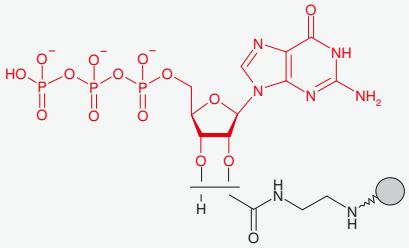
Immobilized Guanosine and m^7-Guanosine Nucleotides

GTP

Linked via the γ-Phosphate

Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
γ-Amino-octyl-GTP AC-106	γ-Phosphate 8-atom carbon spacer 	AC-106S	Bulk material 1 ml	140,—
		AC-106L	Bulk material 5 ml	560,—
		AC-106C	Pre-packed syringe column 1 ml	160,—
		AC-106SC	Pre-packed screening column 0.2 ml	43,—
		AC-106MP	Pre-packed MPLC column 1 ml	640,—
γ-Amino-hexyl-GTP AC-117	γ-Phosphate 6-atom carbon spacer 	AC-117S	Bulk material 1 ml	147,—
		AC-117L	Bulk material 5 ml	588,—
		AC-117C	Pre-packed syringe column 1 ml	168,—
		AC-117SC	Pre-packed screening column 0.2 ml	45,15
		AC-117MP	Pre-packed MPLC column 1 ml	672,—

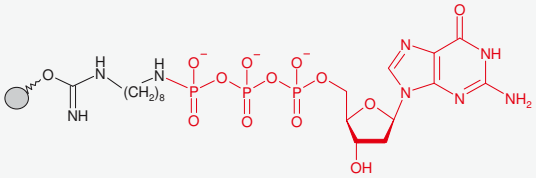
Linked via the Ribose

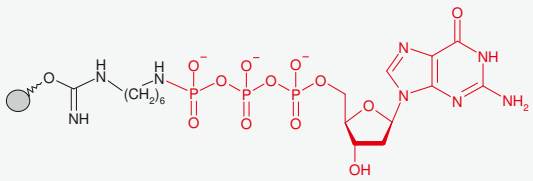
Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
2'/3'-EDA-GTP* AC-132	2'/3'-OH EDA 	AC-132S	Bulk material 1 ml	220,—
		AC-132L	Bulk material 5 ml	880,—
		AC-132C	Pre-packed syringe column 1 ml	240,—
		AC-132SC	Pre-packed screening column 0.2 ml	59,—
		AC-132MP	Pre-packed MPLC column 1 ml	720,—

* Phosphorous chain may be degraded by NTP/dNTP hydrolyzing enzymes.

dGTP

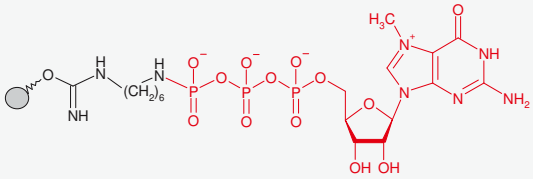
Linked via the γ-Phosphate

Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
γ-Amino-octyl-dGTP AC-112	γ-Phosphate 8-atom carbon spacer 	AC-112S	Bulk material 1 ml	180,—
		AC-112L	Bulk material 5 ml	720,—
		AC-112C	Pre-packed syringe column 1 ml	200,—
		AC-112SC	Pre-packed screening column 0.2 ml	51,—
		AC-112MP	Pre-packed MPLC column 1 ml	680,—

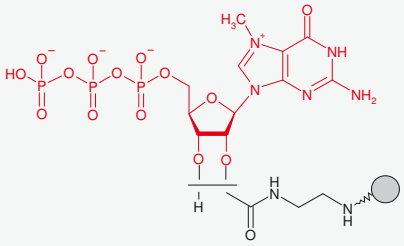
γ-Amino-hexyl-dGTP AC-123	γ-Phosphate 6-atom carbon spacer 	AC-123S	Bulk material 1 ml	180,—
		AC-123L	Bulk material 5 ml	720,—
		AC-123C	Pre-packed syringe column 1 ml	200,—
		AC-123SC	Pre-packed screening column 0.2 ml	51,—
		AC-123MP	Pre-packed MPLC column 1 ml	680,—

m^7GTP

Linked via the γ-Phosphate

Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
γ-Aminohexyl-m^7GTP AC-141	γ-Phosphate 6-atom carbon spacer 	AC-141S	Bulk material 1 ml	180,—
		AC-141L	Bulk material 5 ml	720,—
		AC-141C	Pre-packed syringe column 1 ml	200,—
		AC-141SC	Pre-packed screening column 0.2 ml	51,—
		AC-141MP	Pre-packed MPLC column 1 ml	680,—

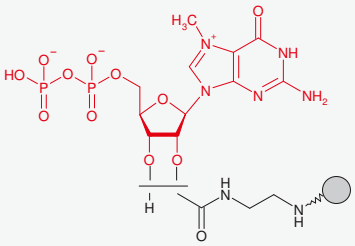
Linked via the Ribose

Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
2'/3'-EDA-m^7GTP* AC-142	2'/3'-OH EDA 	AC-142S	Bulk material 1 ml	180,—
		AC-142L	Bulk material 5 ml	720,—
		AC-142C	Pre-packed syringe column 1 ml	200,—
		AC-142SC	Pre-packed screening column 0.2 ml	51,—
		AC-142MP	Pre-packed MPLC column 1 ml	680,—

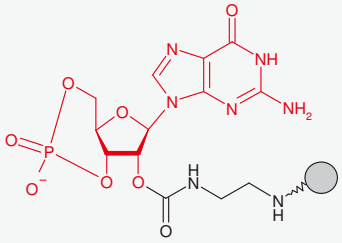
* Phosphorous chain may be degraded by NTP/dNTP hydrolyzing enzymes.

m^7GDP

Linked via the Ribose

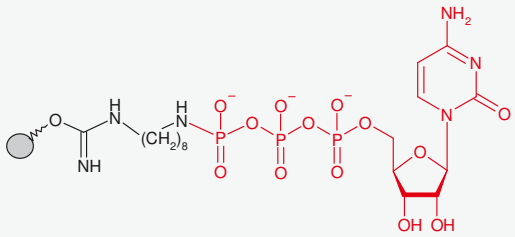
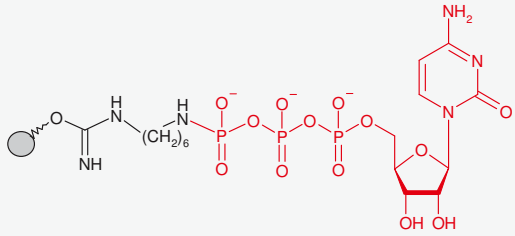
Ligand / Cat.-No.	Attachment Position / Linker Type	Cat.-No.	Type of Material	Price (€)
2'/3'-EDA-m^7GDP AC-143	2'/3'-OH EDA 	AC-143S	Bulk material 1 ml	180,—
		AC-143L	Bulk material 5 ml	720,—
		AC-143C	Pre-packed syringe column 1 ml	200,—
		AC-143SC	Pre-packed screening column 0.2 ml	51,—
		AC-143MP	Pre-packed MPLC column 1 ml	680,—

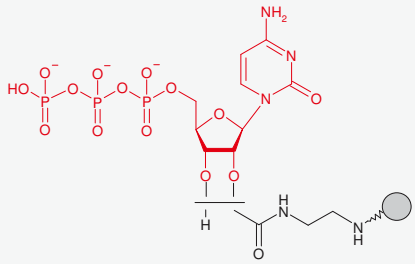
cGMP

Linked via the Ribose				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
2'-EDA-cGMP AC-148	2'-OH EDA 	AC-148S	Bulk material 1 ml	189,—
		AC-148L	Bulk material 5 ml	756,—
		AC-148C	Pre-packed syringe column 1 ml	210,—
		AC-148SC	Pre-packed screening column 0.2 ml	53,55
		AC-148MP	Pre-packed MPLC column 1 ml	714,—

Immobilized Cytidine Nucleotides

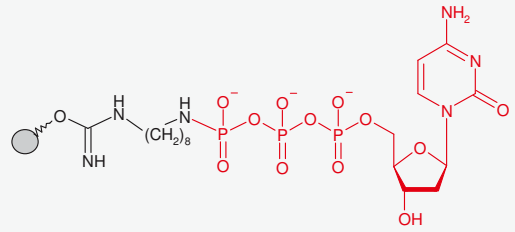
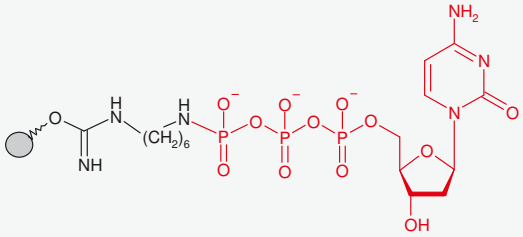
CTP

Linked via the γ-Phosphate				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
γ-Amino-octyl-CTP AC-108	γ-Phosphate 8-atom carbon spacer 	AC-108S	Bulk material 1 ml	140,—
		AC-108L	Bulk material 5 ml	560,—
		AC-108C	Pre-packed syringe column 1 ml	160,—
		AC-108SC	Pre-packed screening column 0.2 ml	43,—
		AC-108MP	Pre-packed MPLC column 1 ml	640,—
γ-Amino-hexyl-CTP AC-119	γ-Phosphate 6-atom carbon spacer 	AC-119S	Bulk material 1 ml	140,—
		AC-119L	Bulk material 5 ml	560,—
		AC-119C	Pre-packed syringe column 1 ml	160,—
		AC-119SC	Pre-packed screening column 0.2 ml	43,—
		AC-119MP	Pre-packed MPLC column 1 ml	640,—

Linked via the Ribose				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
2'/3'-EDA-CTP* AC-134	2'/3'-OH EDA 	AC-134S	Bulk material 1 ml	220,—
		AC-134L	Bulk material 5 ml	880,—
		AC-134C	Pre-packed syringe column 1 ml	240,—
		AC-134SC	Pre-packed screening column 0.2 ml	59,—
		AC-134MP	Pre-packed MPLC column 1 ml	720,—

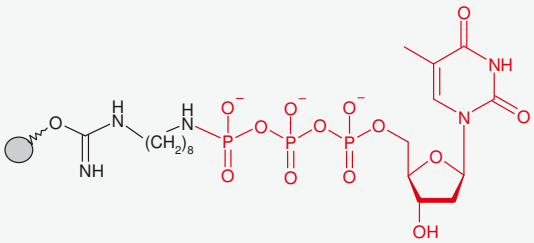
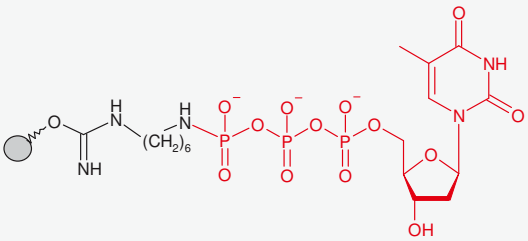
* Phosphorous chain may be degraded by NTP/dNTP hydrolyzing enzymes.

dCTP

Linked via the γ-Phosphate				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
γ-Amino-octyl-dCTP AC-114	γ-Phosphate 8-atom carbon spacer 	AC-114S	Bulk material 1 ml	180,—
		AC-114L	Bulk material 5 ml	720,—
		AC-114C	Pre-packed syringe column 1 ml	200,—
		AC-114SC	Pre-packed screening column 0.2 ml	51,—
		AC-114MP	Pre-packed MPLC column 1 ml	680,—
γ-Amino-hexyl-dCTP AC-125	γ-Phosphate 6-atom carbon spacer 	AC-125S	Bulk material 1 ml	180,—
		AC-125L	Bulk material 5 ml	720,—
		AC-125C	Pre-packed syringe column 1 ml	200,—
		AC-125SC	Pre-packed screening column 0.2 ml	51,—
		AC-125MP	Pre-packed MPLC column 1 ml	680,—

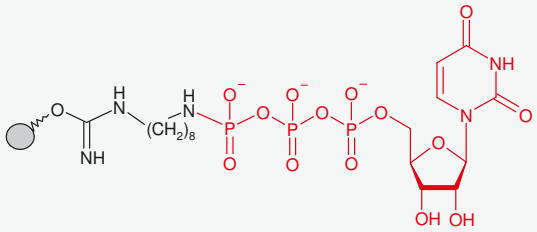
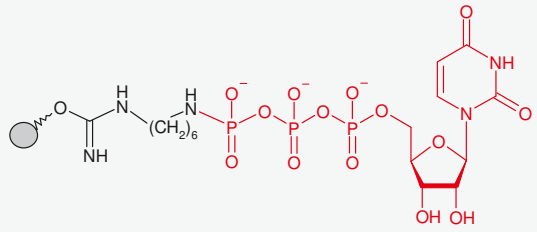
Immobilized Thymidine Nucleotides

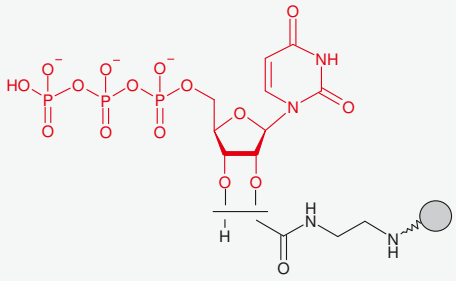
dTTP

Linked via the γ-Phosphate				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
γ-Amino-octyl-dTTP AC-115	γ-Phosphate 8-atom carbon spacer 	AC-115S	Bulk material 1 ml	180,—
		AC-115L	Bulk material 5 ml	720,—
		AC-115C	Pre-packed syringe column 1 ml	200,—
		AC-115SC	Pre-packed screening column 0.2 ml	51,—
		AC-115MP	Pre-packed MPLC column 1 ml	680,—
γ-Amino-hexyl-dTTP AC-126	γ-Phosphate 6-atom carbon spacer 	AC-126S	Bulk material 1 ml	180,—
		AC-126L	Bulk material 5 ml	720,—
		AC-126C	Pre-packed syringe column 1 ml	200,—
		AC-126SC	Pre-packed screening column 0.2 ml	51,—
		AC-126MP	Pre-packed MPLC column 1 ml	680,—

Immobilized Uridine Nucleotides

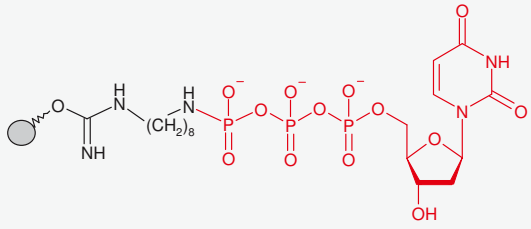
UTP

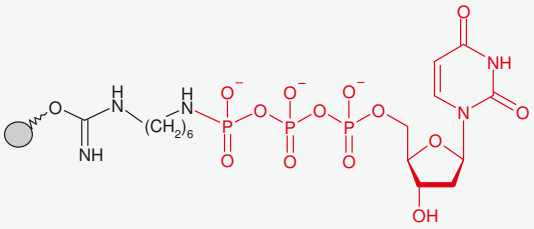
Linked via the γ-Phosphate				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
γ-Amino-octyl-UTP AC-107	γ-Phosphate 8-atom carbon spacer 	AC-107S	Bulk material 1 ml	140,—
		AC-107L	Bulk material 5 ml	560,—
		AC-107C	Pre-packed syringe column 1 ml	160,—
		AC-107SC	Pre-packed screening column 0.2 ml	43,—
		AC-107MP	Pre-packed MPLC column 1 ml	640,—
γ-Amino-hexyl-UTP AC-118	γ-Phosphate 6-atom carbon spacer 	AC-118S	Bulk material 1 ml	140,—
		AC-118L	Bulk material 5 ml	560,—
		AC-118C	Pre-packed syringe column 1 ml	160,—
		AC-118SC	Pre-packed screening column 0.2 ml	43,—
		AC-118MP	Pre-packed MPLC column 1 ml	640,—

Linked via the Ribose				
Ligand	**Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
2'/3'-EDA-UTP* AC-133	2'/3'-OH EDA 	AC-133S	Bulk material 1 ml	220,—
		AC-133L	Bulk material 5 ml	880,—
		AC-133C	Pre-packed syringe column 1 ml	240,—
		AC-133SC	Pre-packed screening column 0.2 ml	59,—
		AC-133MP	Pre-packed MPLC column 1 ml	720,—

* Phosphorous chain may be degraded by NTP/dNTP hydrolyzing enzymes.

dUTP

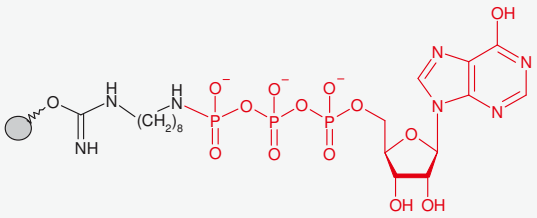
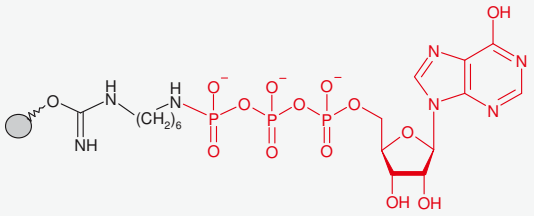
Linked via the γ-Phosphate				
Ligand / Cat.-No.	**Attachment Position / Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
γ-Amino-octyl-dUTP AC-113	γ-Phosphate 8-atom carbon spacer 	AC-113S	Bulk material 1 ml	180,—
		AC-113L	Bulk material 5 ml	720,—
		AC-113C	Pre-packed syringe column 1 ml	200,—
		AC-113SC	Pre-packed screening column 0.2 ml	51,—
		AC-113MP	Pre-packed MPLC column 1 ml	680,—

γ-Amino-hexyl-dUTP AC-124	γ-Phosphate 6-atom carbon spacer 	AC-124S	Bulk material 1 ml	180,—
		AC-124L	Bulk material 5 ml	720,—
		AC-124C	Pre-packed syringe column 1 ml	200,—
		AC-124SC	Pre-packed screening column 0.2 ml	51,—
		AC-124MP	Pre-packed MPLC column 1 ml	680,—

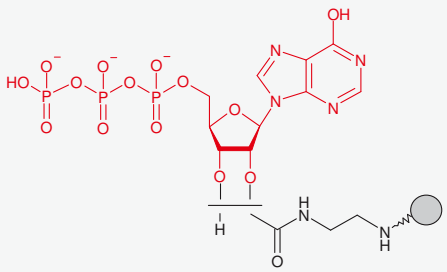
Immobilized Inosine Nucleotides

ITP

Linked via the γ-Phosphate

Ligand	Linker Type	Cat.-No.	Type of Material	Price (€)
γ-Amino-octyl-ITP AC-109	γ-Phosphate 8-atom carbon spacer 	AC-109S	Bulk material 1 ml	180,—
		AC-109L	Bulk material 5 ml	720,—
		AC-109C	Pre-packed syringe column 1 ml	200,—
		AC-109SC	Pre-packed screening column 0.2 ml	51,—
		AC-109MP	Pre-packed MPLC column 1 ml	680,—
γ-Amino-hexyl-ITP AC-120	γ-Phosphate 6-atom carbon spacer 	AC-120S	Bulk material 1 ml	180,—
		AC-120L	Bulk material 5 ml	720,—
		AC-120C	Pre-packed syringe column 1 ml	200,—
		AC-120SC	Pre-packed screening column 0.2 ml	51,—
		AC-120MP	Pre-packed MPLC column 1 ml	680,—

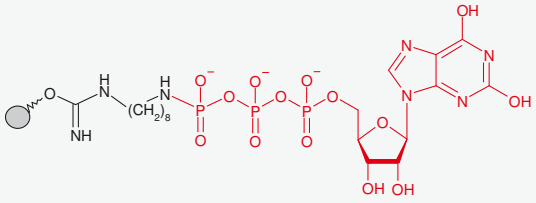
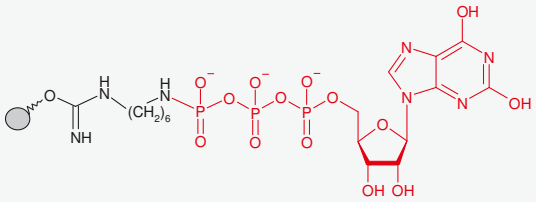
Linked via the Ribose

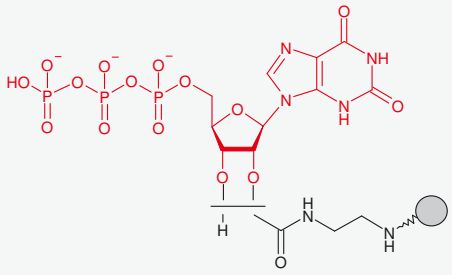
Ligand	Linker Type	Cat.-No.	Type of Material	Price (€)
2'/3'-EDA-ITP* AC-135	2'/3'-OH EDA 	AC-135S	Bulk material 1 ml	220,—
		AC-135L	Bulk material 5 ml	880,—
		AC-135C	Pre-packed syringe column 1 ml	240,—
		AC-135SC	Pre-packed screening column 0.2 ml	59,—
		AC-135MP	Pre-packed MPLC column 1 ml	720,—

* Phosphorous chain may be degraded by NTP/dNTP hydrolyzing enzymes.

Immobilized Xanthosine Nucleotides

XTP

Linked via the γ-Phosphate				
Ligand	**Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
γ-Amino-octyl-XTP AC-110	γ-Phosphate 8-atom carbon spacer 	AC-110S	Bulk material 1 ml	180,—
		AC-110L	Bulk material 5 ml	720,—
		AC-110C	Pre-packed syringe column 1 ml	200,—
		AC-110SC	Pre-packed screening column 0.2 ml	51,—
		AC-110MP	Pre-packed MPLC column 1 ml	680,—
γ-Amino-hexyl-XTP AC-121	γ-Phosphate 6-atom carbon spacer 	AC-121S	Bulk material 1 ml	180,—
		AC-121L	Bulk material 5 ml	720,—
		AC-121C	Pre-packed syringe column 1 ml	200,—
		AC-121SC	Pre-packed screening column 0.2 ml	51,—
		AC-121MP	Pre-packed MPLC column 1 ml	680,—

Linked via the Ribose				
Ligand	**Linker Type**	**Cat.-No.**	**Type of Material**	**Price (€)**
2′/3′-EDA-XTP* AC-136	2'/3'-OH EDA 	AC-136S	Bulk material 1 ml	220,—
		AC-136L	Bulk material 5 ml	880,—
		AC-136C	Pre-packed syringe column 1 ml	240,—
		AC-136SC	Pre-packed screening column 0.2 ml	59,—
		AC-136MP	Pre-packed MPLC column 1 ml	720,—

* Phosphorous chain may be degraded by NTP/dNTP hydrolyzing enzymes.

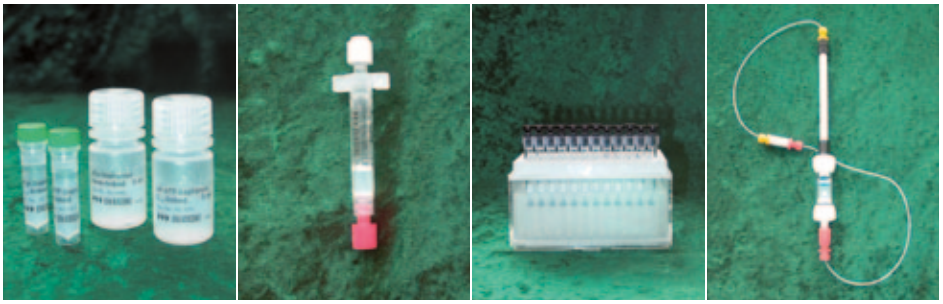
Phospho-Aminoacid binding Proteins

Immobilized phospho-aminoacids provide a convenient and rapid purification procedure for proteins that bind to particular phosphorylated amino acids [1-3].

Jena Bioscience offers affinity chromatography material that is taylor-made for purification (or for related assays) of particular phospho-aminoacid binding proteins since we offer different types and lengths of linkers and provide different types of chromatography material ranging from bulk media to pre-packed columns that fit any machine.

Different types of chromatography material available

All our affinity chromatography materials carrying nucleotides or amino/phospho-amino acids are available as bulk media, pre-packed MPLC columns, syringe columns and screening columns. Please refer to the product pages to access the product lists and data sheets, and to order affinity chromatography products.



Bulk material	Pre-packed syringe column	Pre-packed screening column	Pre-packed MPLC column
based on Agarose (equals Sepharose™ 4B)	based on Agarose (equals Sepharose™ 4B)	based on Agarose (equals Sepharose™ 4B) ▪ maximum amount 0.6 ml ▪ ready-to-use glass columns ▪ one each upper and lower frit ▪ upper and lower end piece ▪ special 96-well-MTP format column holder on request	based on Toyopearl® AF-650M ▪ 40 – 90 µ material ▪ 1× Omnifit column kit 10 × 50 mm ▪ 1× adjustable end piece ▪ 2× female-female (1/4" to 6 mm) coupling ▪ 2 m PTFE tubing (1/16" OD x 0.8 mm ID) MPLC columns are delivered with adapters for FPLC Systems of BioRad, GE Healthcare (Äkta™) and Pharmacia.

Toyopearl® is a trademark of TOSOH Bioscience
Äkta™ is a trademark of GE Healthcare Companies

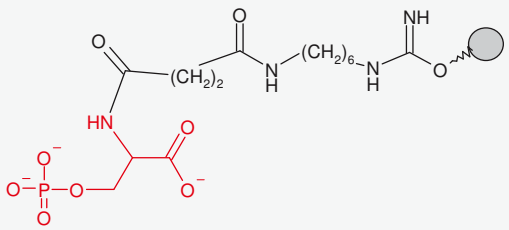
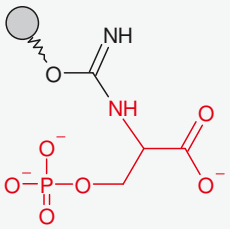
Selected References:

[1] Bakalara *et al.* (2000) Purification, Cloning, and Characterization of an Acidic Ectoprotein Phosphatase differentially expressed in the infectious bloodstream form of *trypanosoma brucei*. *J. Biol. Chem.* **275 (12)**:8863.

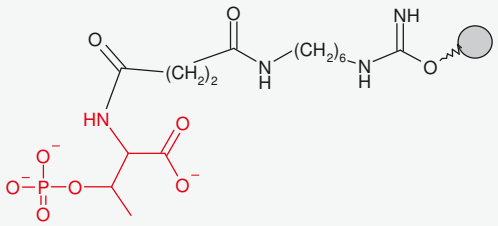
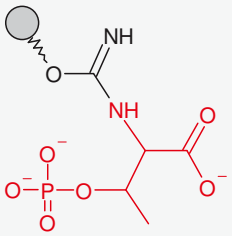
[2] Muir *et al.* (1998) Expressed protein ligation: A general method for protein engineering. *Proc. Natl. Acad. Sci. USA* **95**:6705.

[3] Pei *et al.* (1996) Phospholipase C-y1 binds to actin-cytoskeleton via its C-terminal SH2 domain *in vitro*. *Biochem. Biophys. Res. Comm.* **228**:802.

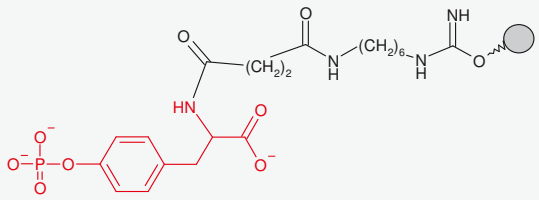
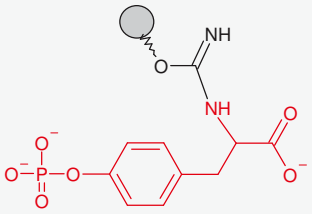
Immobilized O-Phospho-Serine

Linker Type	Cat.-No.	Type of Material	Price (€)
C_{10}-spacer 	AC-137S	Bulk material 1 ml	80,—
	AC-137L	Bulk material 5 ml	320,—
	AC-137C	Pre-packed syringe column 1 ml	110,—
	AC-137SC	Pre-packed screening column 0.2 ml	31,—
	AC-137MP	Pre-packed MPLC column 1 ml	580,—
no spacer 	AC-138S	Bulk material 1 ml	50,—
	AC-138L	Bulk material 5 ml	200,—
	AC-138C	Pre-packed syringe column 1 ml	80,—
	AC-138SC	Pre-packed screening column 0.2 ml	25,—
	AC-138MP	Pre-packed MPLC column 1 ml	550,—

Immobilized O-Phospho-Threonine

Linker Type	Cat.-No.	Type of Material	Price (€)
C_{10}-spacer 	AC-139S	Bulk material 1 ml	80,—
	AC-139L	Bulk material 5 ml	320,—
	AC-139C	Pre-packed syringe column 1 ml	110,—
	AC-139SC	Pre-packed screening column 0.2 ml	31,—
	AC-139MP	Pre-packed MPLC column 1 ml	580,—
no spacer 	AC-140S	Bulk material 1 ml	50,—
	AC-140L	Bulk material 5 ml	200,—
	AC-140C	Pre-packed syringe column 1 ml	80,—
	AC-140SC	Pre-packed screening column 0.2 ml	25,—
	AC-140MP	Pre-packed MPLC column 1 ml	550,—

Immobilized O-Phospho-Tyrosine

Linker Type	Cat.-No.	Type of Material	Price (€)
C_{10}-spacer 	AC-103S	Bulk material 1 ml	80,—
	AC-103L	Bulk material 5 ml	320,—
	AC-103C	Pre-packed syringe column 1 ml	110,—
	AC-103SC	Pre-packed screening column 0.2 ml	31,—
	AC-103MP	Pre-packed MPLC column 1 ml	580,—
no spacer 	AC-104S	Bulk material 1 ml	50,—
	AC-104L	Bulk material 5 ml	200,—
	AC-104C	Pre-packed syringe column 1 ml	80,—
	AC-104SC	Pre-packed screening column 0.2 ml	25,—
	AC-104MP	Pre-packed MPLC column 1 ml	550,—

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