DGM

Density Gradient Media

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serumwerk bernburg

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Pharmaceutical diversity made in Germany

Health is particularly close to our hearts

Serumwerk Bernburg was founded in 1954 and has been operating as a public limited company since 1992. Over the past decades, we have established ourselves as a globally recognised producer and supplier for human and veterinary medicine. At the same time, we are strengthening the region in a sustainable manner through our variety of jobs and economic stability.

Together with our subsidiaries, we cover a broad portfolio of pharmaceutical products in different dosage forms.

A special focus of Serumwerk Bernburg AG is on intravenous iron preparations, especially for the treatment of anaemia in suckling piglets. We are worldwide one of the two leading companies in this field.

In human medicine, we are also developing a highly innovative intravenous iron preparation, which we expect will also reach a top position worldwide.

Lymphoprep

Isolation of human mononuclear cells

A simple and effective method for the isolation of mononuclear cells from human blood was reported by Dr. Arne Bøyum in 1968. For more than 45 years a commercial medium known as **Lymphoprep** has been widely used for isolating these cells.

Mononuclear cells (monocytes and lymphocytes) have a lower buoyant density than the erythrocytes and the polymorphonuclear (PMN) leukocytes (granulocytes). The vast majority of mononuclear cells have densities below 1.077 g/ml.

These cells can therefore be isolated by centrifugation on an isoosmotic medium with a density of 1.077 g/ml, which allows the erythrocytes and the PMNs to sediment through the medium while retaining the mononuclear cells at the sample/medium interface.

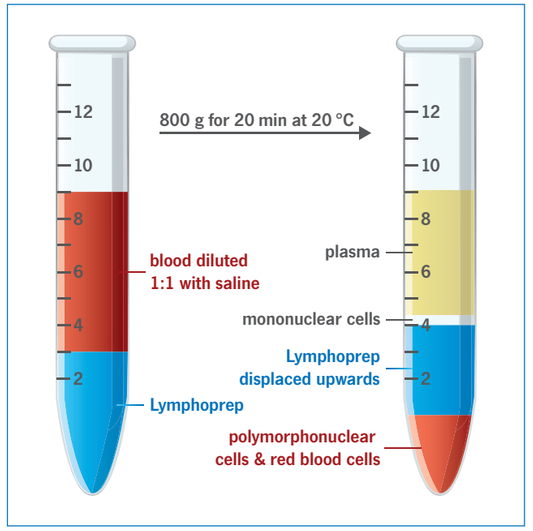
The described method is rapid, simple and reliable and gives excellent results with blood samples.

To obtain the maximum yield it is important that the blood sample is diluted 1:1 with physiological saline before being applied to the gradient.

The contamination of erythrocytes in the mononuclear cell suspension is usually between 3-10 % of the total cell number.

Some immature PMNs may band with the lymphocytes during intense immunosuppressive therapy.

When heparinised blood is used, it is essential to remove most of the platelets, in order to avoid inhibition in the cytotoxicity test.



Each batch of **Lymphoprep** is checked on the level of endotoxins using a specific LAL test. Our goal is to produce batches with an endotoxin level lower or equal to 0.13 IU/ml.

For every batch produced a Certificate of Analysis showing the actual values of density, osmolality and endotoxins is made available at **"diagnostic.serumwerk.com"**. We also claim sterility according to Ph.Eur.

Lymphoprep is manufactured, packed and released by a GMP compliant and ISO 13485 certified manufacturer.

Lymphoprep has the same specifications as the expensive PLUS or PREMIUM media from other manufacturers.

Lymphoprep can be used for the preparation of pure lymphocyte suspensions, antilymphocyte sera and immunological research. Erik Thorsby and Anne Bratlie used this technique with only slight modifications in the preparation of pure lymphocyte suspensions for cytotoxicity tests and lymphocyte cultures.

Lymphoprep is a ready-made, sterile and endotoxin tested solution with the following specifications:	
Sodium diatrizoate	9.1 % (w/v)
Polysucrose 400	5.7 % (w/v)
Density	1.077 ± 0.001 g/ml
Osmolality	290 ± 15 mOsmol/kg
Endotoxins	< 1.0 IU/ml

Lymphoprep is supplied as a sterile solution in the following package sizes:	
Prod. No. 1856	1 x 250 ml (available as single bottle or as set containing 4 bottles)
Prod. No. 1858	1 x 500 ml (available as single bottle or as set containing 6 bottles)

References:

Bøyum, A. (1968)
Separation of leucocytes from blood and bone marrow Scand. J. Clin. Lab. Invest., 21, suppl.97

Lymphoprep Tube

Isolation of human mononuclear cells – easy handling

A simple and effective method for the isolation of mononuclear cells from human blood was reported by Dr. Arne Bøyum in 1968. For more than 45 years a commercial medium known as **Lymphoprep** has been widely used for isolating these cells.

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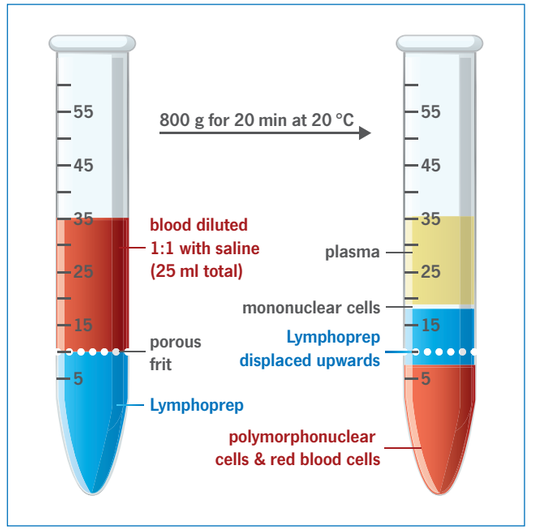
These cells can therefore be isolated by centrifugation on an isoosmotic medium with a density of 1.077 g/ml, which allows the erythrocytes and the PMNs to sediment through the medium while retaining the mononuclear cells at the sample/medium interface.

The described method is rapid, simple and reliable and gives excellent results with blood samples.

The success of the standard method for isolation mononuclear cells using **Lymphoprep** depends to a large extent on the careful layering of the diluted blood sample on top of the centrifugation medium to maintain a sharp interface between the two layers. This procedure requires some practise and can be time-consuming with large numbers of samples.

Lymphoprep Tube is a sterile tube in which the **Lymphoprep** is contained below a porous frit.

This allows diluted blood to be poured simply and directly into the tube, the disc preventing any mixing with the separation medium.



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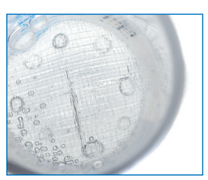
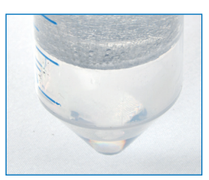
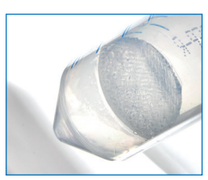
Polysucrose 400	5.7 % (w/v)
Density	1.077 ± 0.001 g/ml
Osmolality	290 ± 15 mOsmol/kg
Endotoxins	< 1.0 IU/ml

Lymphoprep Tube is supplied as a sterile solution in the following package sizes:	
Prod. No. 18002	30 x 2 ml (**Lymphoprep** volume) filled in in conical centrifuge tubes (total tube capacity 15 ml)
Prod. No. 18001	18 x 10 ml (**Lymphoprep** volume) filled in in conical centrifuge tubes (total tube capacity 50 ml)

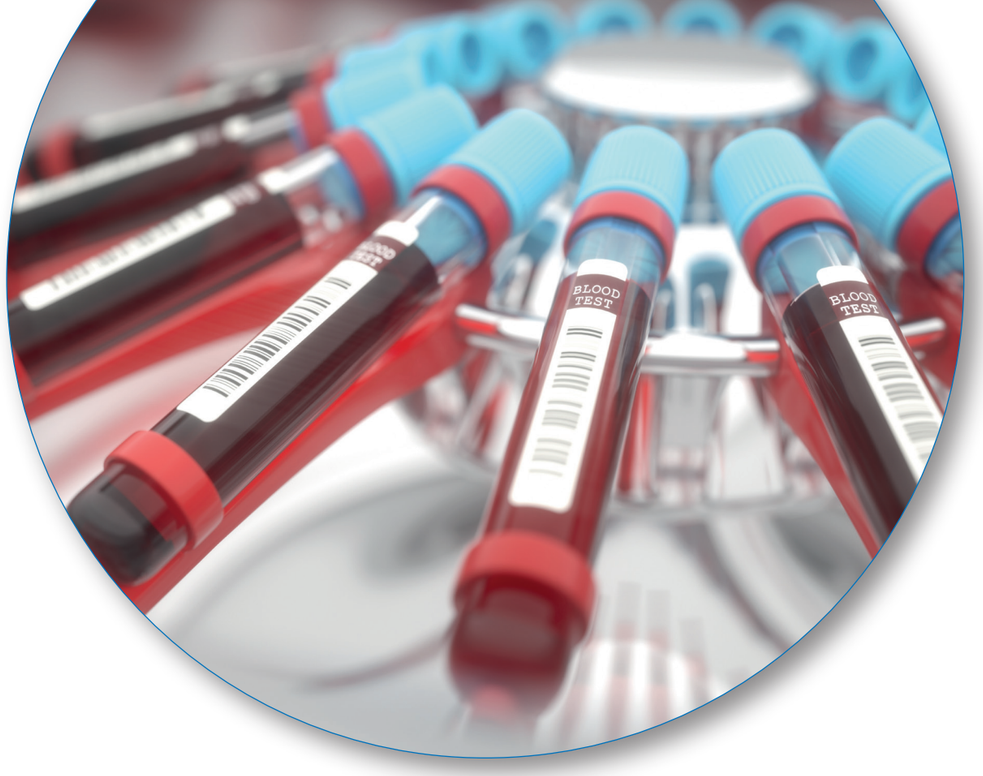






References:

Bøyum, A. (1968)
Separation of leucocytes from blood and bone marrow Scand. J. Clin. Lab. Invest., 21, suppl.97



OptiPrep

The optimum density gradient medium

OptiPrep is a sterile and endotoxin tested solution of 60 % iodixanol in water with a density of 1.320 g/ml. **Iodixanol** was developed as an X-ray contrast medium an has therefore been subjected to rigorous clinical testing. It is non-ionic, non-toxic to cells and metabolically inert. **Iodixanol** solutions can be made isoosmotic at all useful densities, this solutions have low viscosity and osmolality.

The high density of **OptiPrep** facilitates the fractionation of cells by flotation from a dense load zone through either a continuous or discontinuous gradient or through a simple density barrier.

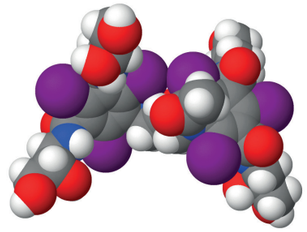
- **51 protocols available for cell isolation.**

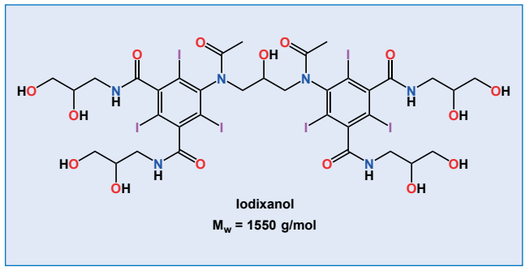
Improved resolution of cell organelles. Low viscosity, isoosmotic gradients provide rapid and efficient separation of the major organelles in preformed gradients.

- **62 protocols available for isolation of subcellular organelles and membranes.**

OptiPrep is the ideal solution for virus purification. Virus purified using **OptiPrep** gradients shows infectivity: particle number ratios at least 100x those from CsCl gradients.

- **38 protocols available for virus purification**
- **13 protocols available for macromolecules and lipoproteins purification.**





Each batch of **OptiPrep** is checked on the level of endotoxins using a specific LAL test. Our goal is to produce batches with an endotoxin level lower or equal to 0.13 IU/ml.

For every batch produced a Certificate of Analysis showing the actual values of density and endotoxin levels is made available at **"diagnostic.serumwerk.com"**. We also claim sterility according to Ph.Eur.

OptiPrep is manufactured, packed and released by a GMP compliant and ISO 13485 certified manufacturer.

OptiPrep is a ready-made, sterile and endotoxin tested solution with the following specifications:	
Iodixanol	60 % (w/v)
Density	1.320 ± 0.001 g/ml
Endotoxins	< 1.0 IU/ml

OptiPrep is supplied as a sterile solution in the following package sizes:	
Prod. No. 1893	1 x 250 ml

Applications

Isolation of:

- Mammalian and non-mammalian cells
- Subcellular organelles
- Plasma membranes and domains
- Membrane vesicles and cytosol
- Organelles from non-mammalian sources
- Viruses
- Plasma lipoproteins
- Proteins and protein complexes
- Plasmid DNA
- Ribonucleoproteins

Analysis of:

- Membrane trafficking and cell signalling
- Endocytosis and exocytosis

Polymorphprep

Isolation of human polymorphonuclear cells

With the exception of the basophils, polymorphonuclear leukocytes (PMNs) have a much greater buoyant density than the mononuclear cells, >1.085 g/ml. Unfortunately, the buoyant density of the erythrocytes tends to be from 1.09 – 1.11 g/ml, this makes a separation from whole blood using a density barrier similar to that used for mononuclear cells more difficult. A number of procedures have been developed in an effort to overcome these difficulties.

The high osmolality of **Polymorphprep** causes erythrocytes to lose water and shrink, thus increasing their effective buoyant densities. This allows the aggregated erythrocytes to sediment rapidly through the dense medium.

Each batch of **Polymorphprep** is checked on the level of endotoxins using a specific LAL test. Our goal is to produce batches with an endotoxin level lower or equal to 0.13 IU/ml.

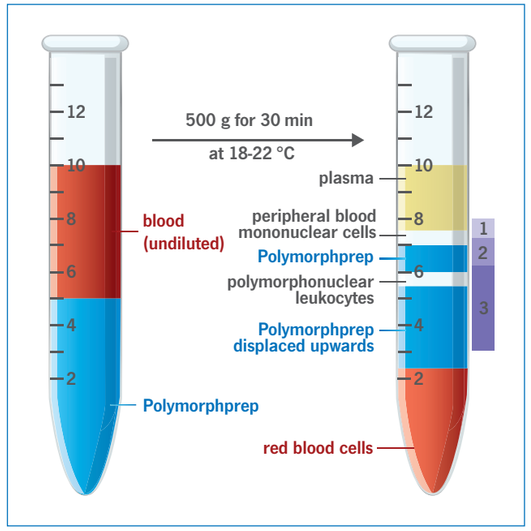
The method is effective only with whole undiluted human blood not with a leukocyte-rich fraction or blood from animal species.

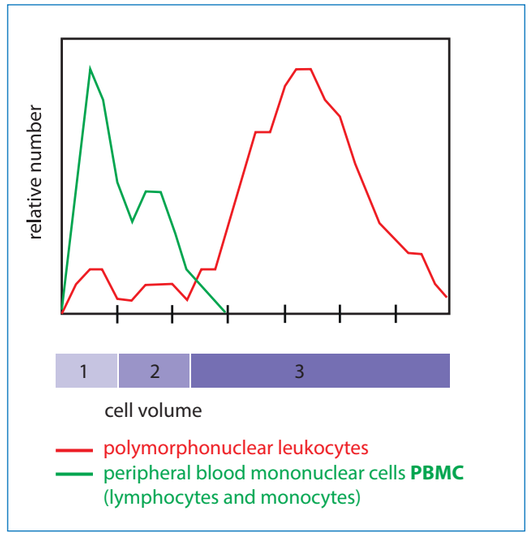
The temperature is important to obtain optimal results, as changes in temperature effect the density and viscosity of the **Polymorphprep** solution. The temperature of the blood sample and the medium should be kept between 18-22 °C.

Analysis of the top and bottom bands from the **Polymorphprep** separation using a Coulter STKR Cell Analyser is shown in the figure on the right. The analyzer determines the number of cells in the sample (ordinate) as a function of cell volume (abscissa). Relative cell number is the number of cells of a particular volume expressed as a fraction of the total in each sample.

The cell band on top of the **Polymorphprep** contains only peripheral blood mononuclear cells (PBMCs). The cell band itself can be separated into lymphocytes (upper layer, 1) and monocytes (lower layer, 2).

All of the PMNs are in the bottom cell band, which is enclosed of the **Polymorphprep** solution. Contamination of the PMN band by erythrocytes is between 2-6 % of the total cell number.

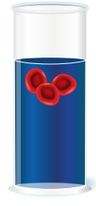




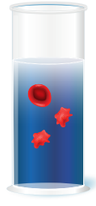
Polymorphprep is a ready-made, sterile and endotoxin tested solution with the following specifications:	
Sodium diatrizoate	13.8 % (w/v)
Polysucrose 400	8.0 % (w/v)
Density	1.113 ± 0.001 g/ml
Osmolality	445 ± 15 mOsmol/kg
Endotoxins	< 1.0 IU/ml

Polymorphprep is supplied as a sterile solution in the following package sizes:	
Prod. No. 1895	1 x 250 ml

Behaviour of Erythrocytes during centrifugation with Polymorphprep



A
starting condition:
Erythrocytes (red blood cells) are surrounded by Polymorphprep, which has a higher osmolality (hypertonic solution) than the cells.



B
during centrifugation:
Due to the high osmolality the erythrocytes loose water and shrink. The effective buoyant density of the cells is simultaneously decreasing enabling further sedimentation.

C
end conditions:
The osmotic gradient between the erythrocytes and Polymorphprep is balanced.

Nycodenz

A universal density gradient medium

Nycodenz is an off-white powder, freely soluble in water. Solution up to 80 % (w/v) with a density up to 1.426 g/ml can be prepared.

It was originally developed as an X-ray contrast medium and have therefore been subjected to rigorous clinical testing.

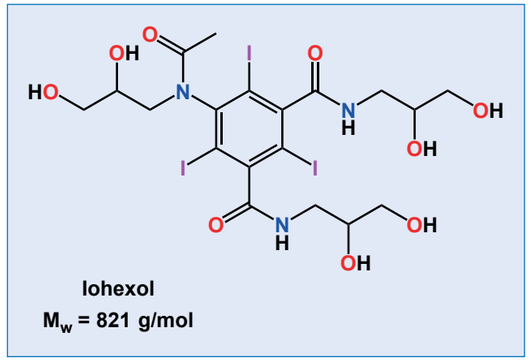
- non-ionic, non-toxic to cells and metabolically inert
- can be used for the isolation of cells, subcellular organelles and membranes, macromolecules and viruses
- forms true solutions. It is therefore easy to remove the medium from the cells after fractionation
- is resistant to bacterial degradation
- solutions can be autoclaved

Nycodenz is the trademark name for iohexol, whose systematic name is
5-(N-2,3-dihydroxypropylacetamido)-2,4,6-tri-iodo-N-N'-bis(2,3-dihydroxypropyl)isophthalamide.
It has a molecular weight of 821 g/mol. The chemical properties and stability of **Nycodenz** are related to its structure.

Its high density derives from the presence of a substituted triiodobenzene ring linked to a number of hydrophilic groups which are responsible for the high water solubility of **Nycodenz**. It is a non-ionic derivative of metrizoic acid; the carboxyl group present in metrizoic acid is linked to the amine group of 3-amino-1,2-propanediol. The dihydroxypropylacetamido side chain is responsible for the very low toxicity of **Nycodenz** compared to metrizamide. **Nycodenz** has a defined melting point between 174 and 180 °C. The iodinated aromatic nucleus absorbs strongly in the ultraviolet region of the spectrum with an absorbtion maximum of 244 nm

Gradients of Nycodenz can be generated by:

- centrifugation **in situ** (self-forming gradients).
- diffusion. Using **Nycodenz**, linear gradients can be simply prepared within 45 minutes.
- freezing and thawing.
- tilted tube rotation (Gradient Master™).



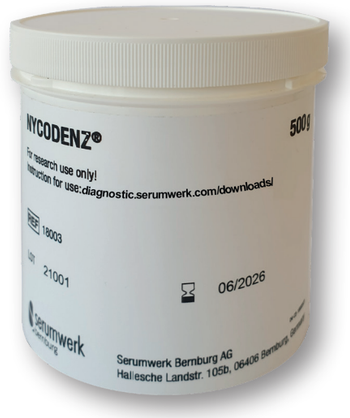
The density of **Nycodenz** in solution can be determined by measuring the refractive index. The density can also be determined spectrophotometrically.

Nycodenz is a non-particulate medium therefore the distribution of cells in gradients can be determined using a haemocytometer, electronic particle counter or by light scattering measurements using a spectrophotometer.

Nycodenz does not interfere with the orcinol and diphenylamine reactions for the estimation of nucleic acids nor with the very sensitive dye binding assays for protein and DNA.

Polysaccharides and sugars can be determined in the presence of **Nycodenz** using the phenol/H_2SO_4 assay. Fluorimetric assays of nucleic acids and proteins can also be carried out in the presence of **Nycodenz**. **Nycodenz** does not interfere with most assays for the marker enzymes of subcellular components, also commercial scintillants are compatible with **Nycodenz**.

Nycodenz can be removed from samples by dialysis, ultrafiltration or gel filtration. Cells, subcellular organelles and other particulate matter can be isolated from **Nycodenz** by centrifugation without the risk of contaminating the pellet with **Nycodenz**.



Nycodenz is supplied as a powder in the following package size:	
Prod. No. 18003	1 x 500 g

Applications

Isolation of:

- Mammalian and non-mammalian cells
- Subcellular organelles
- Organelles from non-mammalian sources
- Subcellular membranes
- Protein and protein complexes
- Ribonucleoproteins
- Viruses

Polysucrose 400

A universal density gradient medium

Polysucrose 400 is a synthetic high molecular weight polymer made by the copolymerization of sucrose and epichlorohydrin. The molecules have a branched structure with a high content of hydroxyl groups giving a good solubility in aqueous solutions.

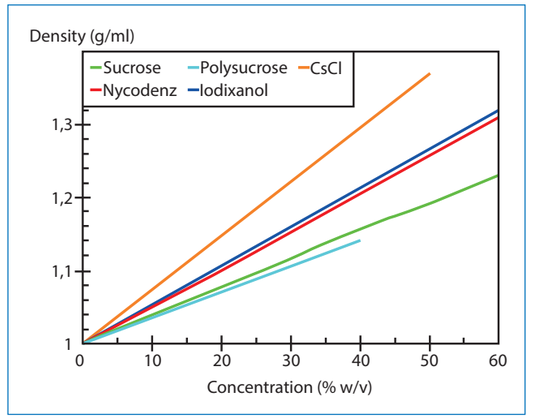
The reactivity and stability of **Polysucrose 400** are determined by its hydroxyl groups and the glycosidic bonds in the sucrose residues. **Polysucrose 400** is stable in alkaline and neutral solutions. At pH values lower than 3, it is rapidly hydrolysed, especially at elevated temperatures. In neutral solutions, **Polysucrose 400** can be sterilized by autoclaving at 110 °C for 30 minutes without any degradation.

Polysucrose 400 is readily soluble in aqueous solutions when added slowly to the liquid with constant stirring. Concentrations up to 50 % (w/v) can easily be obtained.

Applications

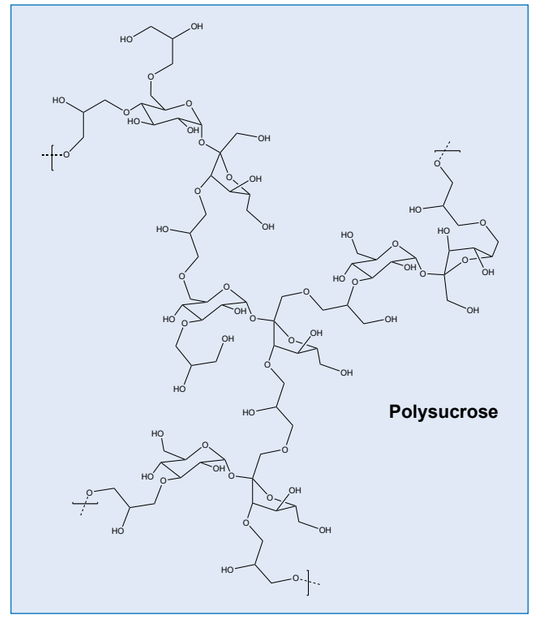
Using sodium metrizoate and a polysaccharide Bøyum (1968) developed a one-step centrifugal technique for isolation of lymphocytes (Lymphoprep). In this method the polysaccharide aggregates the erythrocytes, thereby increasing their sedimentation rate. **Polysucrose 400** has also been used as a density gradient medium for the purification of other cells and in membrane fractionation.

Non-ionic high molecular weight solutes such as polysucrose are required for a number of other research scenarios. **Polysucrose 400** may be used as a stabilizing agent in protein solutions and it can function as an immunologically inert carrier for low molecular weight haptens in immunological studies. **Polysucrose 400** is also used to reduce non-specific binding of labelled probes to nitrocellulose membranes during nucleic acid hybridization. It also simplifies the loading of nucleic acids into the sample wells of agarose gels for electrophoresis.



Technical data	
White odourless powder	
Absorption	< 0.45
Specific optical rotation $(\alpha)_d^{20}$	53° – 59°
Intrinsic viscosity (20 °C)	0.14 – 0.20
Average molecular weight (Mw)	450 ± 100 kDa
Mw distribution by GPC	conforms to standard
Loss on drying (%)	max. 5.0 %
pH (10 % w/v aqueous solution)	7.0 – 9.0
Sulphated ash	max. 0.3 %
Content of chloride (ppm)	max. 500 ppm
Sterilization test	conforms
Microbiological contamination	max. 100 CFU/g max. 10 yeasts and mould/g
Bacterial endotoxins	max. 10.0 IU/g

Polysucrose 400 is available in the following package sizes:	
Prod. No. 7828	20 kg



References:

Bøyum, A. (1968)
Separation of leucocytes from blood and bone marrow Scand. J. Clin. Lab. Invest., 21, suppl.97

Products

Lymphoprep

size:	250 ml	500 ml
prod. no.	1856	1858

Lymphoprep Tube

size:	30 x 2 ml	18 x 10 ml
prod. no.	18002	18001
total tube size:	15 ml	50 ml

OptiPrep

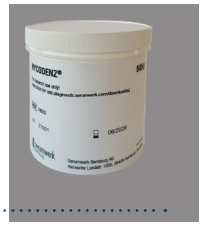
size:	250 ml
prod. no.	1893

Polymorphprep

size:	250 ml
prod. no.	1895

Nycodenz

size:	500 g
prod. no.	18003



Polysucrose 400

size:	20 kg
prod. no.	7828

serumwerk bernburg

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