

## Rabbit anti-Human SSEA-4 Antibody

Product #: GKGF8RBIGG100

Clone name: E2-F8

Product type: Recombinant rabbit IgG monoclonal antibody

Lot number: T2233A17

**Source:** Cell culture expression in HEK293 cells

**Purification:** Purified by Protein A or Protein G affinity chromatography

Immunogen: SSEA4

**Buffer:** Phosphate buffered saline pH7.5

**Endotoxin:** <0.050 EU/mg as determined by LAL chromogenic endotoxin assay

Concentration: 1mg/ml

Vial size: 0.1mg

## **Target information:**

Stage-specific embryonic antigen-4 (SSEA-4) is a ganglioside which consists of a glycosphingolipid containing a terminal sialic acid residue (N-acetylneuraminic acid). SSEA-4 expression changes both qualitatively and quantitatively during development, differentiation and in tumorigenesis. During human development, SSEA-4 is first observed on pluripotent cells of the inner cell mass and is subsequently lost upon differentiation (Tondeur et al. 2008). In addition, human germ stem cells in the testis and ovary express SSEA-4 (Harichandan et al. 2013; Virant-Klun et al. 2013).

SSEA-4 has been found to be over-expressed in a number of cancers and has been correlated with disease progression. Studies by the Buhring group proposed SSEA-4 as a novel marker to identify heterogeneous, invasive subpopulations of tumor cells. Importantly, they also demonstrated that increased cell-surface SSEA-4 expression is associated with the loss of cell—cell interactions and the gain of a migratory phenotype, suggesting an important role of SSEA-4 in cancer invasion by influencing cellular adhesion to the extracellular matrix.

Studies of SSEA-4 expression and function have often been inhibited by the lack of high quality reagents. Due to the glycan nature of the antigen almost all antibodies that have been developed are of IgM or IgG3 classes, which tend to be of low affinity and can be difficult to handle.

Glykogen have overcome these reagent challenges by using their proprietary immunisation methods to develop Clone F8, a recombinant monoclonal rabbit IgG antibody. The availability of this antibody will facilitate studies into SSEA-4 expression and function, both in stem cell biology and in cancer studies.

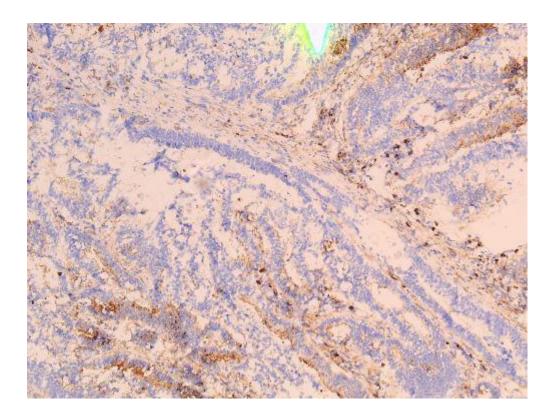
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The chemical structure of the SSEA-4 antigen is shown below

## **Applications:**

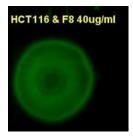
**Immunohistochemistry:** This antibody may be used to stain frozen tissue sections. A dilution range of 40 ug/ml is suggested, but users should titrate the antibody for best results within their own systems.

The image below shows staining of fresh frozen human colon cancer tissue, demonstrating antibody binding to individual cells and to discrete stromal components.



**Immunoblotting:** This antibody may be used for immunoblotting of lipid extracts of cells. A dilution range of 10-50 ug/ml is suggested, but users should titrate the antibody for best results within their own systems. NB: Due to the glycan nature of the epitope recognised by this antibody, traditional protein Western Blotting cannot be used.

The image below shows lipid immunoblotting of a total lipid extract of HCT116 cells using the F8 antibody at 40ug/ml (with Sigma A0545 Goat anti-rabbit IgG peroxidase).



**ELISA:** This antibody may be used in ELISA applications. A dilution range of 30-50 ug/ml is suggested, but users should titrate the antibody for best results within their own systems

**Recommended secondary antibody:** A suitable anti-rabbit IgG reagent such as product A0545 (Goat anti-rabbit IgG:HRP) or SAB3700963 (Donkey anti-rabbit IgG (H+L) F(ab')<sub>2</sub>:FITC from Sigma is recommended

**Shipping:** The product is shipped at ambient temperature. Upon receipt store immediately as recommended below

**Storage:** Use a manual defrost freezer and avoid repeated freeze/thaw cycles. Store frozen in aliquots at -20°C for up to one year, or at 4°C for up to one week

## References

1. Sivasubramaniyan, K., Harichandan, A., Schilbach, K., Mack, A. F., Bedke, J., Stenzl, A., Kanz, L., Niederfellner, G., & Bühring, H. J. (2015). Expression of stage-specific embryonic antigen-4 (SSEA-4) defines spontaneous loss of epithelial phenotype in human solid tumor cells. *Glycobiology*, *25*(8). https://doi.org/10.1093/glycob/cwv032

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