



Product Specification Sheet

Keyhole limpet Hemocyanin (KLH)-Agarose (affinity matrix)

Cat # KLH11-G,
Cat # KLH11-G-5

KLH-Agarose
KLH-Agarose

SIZE: 1 ml

SIZE: 5 ml

Antibodies to small peptide/haptens are generally raised by coupling to a large carrier proteins such as KLH. Antibodies are produced to both KLH and the peptide/hapten. Anti-KLH may give non-specific signals in various immunoassays. Anti-KLH antibodies can be removed by solid phase immunoaffinity column chromatography over the KLH-Agarose.

Source

KLH, isolated from the hemolymphs of the mollusk *Megathura crenulata*, belongs to a large family of giant respiratory proteins called hemocyanins, which are found in mollusks and arthropods. KLH was coupled to CNBR-activated Sepharose 4B at 3-4 mg protein/ml.

Form & Storage

The product is supplied as 1 ml settled gel in 1 ml of 0.01 PBS, 7.4 and 0.1% sodium azide (50% gel suspension) in a 2 ml bed volume chromatography column. Do not freeze. Store at 4°C.

Binding capacity

Process 2-5 ml of high titer antiserum or 10 mg IgG over 1 ml of KLH-Agarose. The unbound fraction may be re-processed if necessary. The column can be used many times following standard binding, elution, and regeneration conditions.

General References: Harlow E and Lane D (1988) Antibodies: A lab, manual

How to use the Affinity Column

The KLH-agarose is supplied in 2-ml plastic column that is capped at the bottom. Break-off the flat tip to allow the buffer drain and allow sample flow. A yellow cap is also provided to cap the column when not in use or stop the column. It is also possible to cap the column with parafilm if the yellow cap is lost.

A general protocol for removal of KLH antibodies

Anti-KLH antibody removal can be performed using a small column (supplied) or the batch process. Purification can be performed at 4°C (for temp sensitive proteins) or room temp.

1. Dilute 2-5 ml antiserum 1:2 with PBS azide and apply to the column and recycle 2-3 times. Anti-KLH will bind to the column and the remaining antiserum collected in the flow through.

The adsorbed antiserum (flow through) should be compared with the unprocessed antiserum to confirm the removal of anti-KLH. If necessary, recycle the antiserum on fresh column or recycled column.

Regeneration of the column

Apply 10-20 ml of elution buffer (0.1M Gly-HCL pH 2.5), followed by 20-30 mls of PBS, pH 7.4 and 0.01% azide. Store recycled column at 4°C.

Other Fusion tag antibodies available from ADI

KLH11-G Keyhole Limpet Hemocyanin (KLH)-Sepharose affinity gel for removing KLH antibodies

BSA and Ovalbumin-Agarose also available.

KLH12-M monoclonal Anti-KLH (keyhole leimpet hemocyanin)
KLH13-S Anti-KLH (keyhole limpet hemocyanin) antiserum #3
KLH14-S Anti-KLH (keyhole limpet hemocyanin) antiserum #4
KLH15-S Anti-KLH (keyhole limpet hemocyanin) antiserum #5
KLH51-R10 Keyhole Limpet Hemocyanin (*Megathura Crenulata*) Carrier protein

Anti-MBP, Poly-His, GST, beta-Gal, VSV-G, Flag, HA-tag, and c-myc

Anti-Rabbit IgG-HRP Conjugate and ECL Reagents

Western Blot Recycling Kit (Strips blots in 5 minutes) and re-use the same blot with multiple antibodies

KLH11-G-5

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