



Product Specification Sheet

Anti-HA-tag Agarose

Cat. # HA12-AS

Goat Anti-HA tag IgG-Agarose

SIZE: 250 ul

FORM:

Soln

Lyophilized

Expression of genes in E. coli or yeast or baculovirus offers a convenient system to produce large amounts of recombinant proteins that may otherwise be difficult to isolate from natural cells and tissues. Very often antibodies to these newly identified proteins are not available to study its biochemical properties, monitor protein expression, and purification. In order to circumvent this problem, short pieces of well-defined peptides (Poly-His, Flag-epitope or c-myc epitope or HA-tag) or small proteins (bacterial GST, MBP, Thioredoxin, b-Galactosidase, VSV-Glycoprotein etc) are often cloned along with the target gene. Proteins are expressed as fusion proteins. Antibodies to these fusion-tags are already available to monitor fusion protein expression and purification. Therefore, fusion-tags serve as universal tags much like secondary antibodies. Many tags have their own characteristics. Poly-His-fusion proteins (6 x His) can bind to Nickel-Sepharose or Nickel-HRP. GST-fusion proteins can bind to glutathione-Sepharose. Therefore, a high degree of purification of fusion protein can be achieved in just one affinity purification step. Purity of fusion proteins can be followed by Tag-antibodies. Very often, fusion proteins are directly injected into animals to generate antibodies. Some fusion tags can be removed later by treatment with enzymes to generate tag-free recombinant proteins.

Source of Antigen and Antibodies

A 9-aa peptide sequence (**designated cat # HA12-P; control peptide**) from hemagglutinin influenza virus (aa 114-122; YPY DVP DYA) (1) was synthesized and purified by HPLC. HA12-P was coupled to KLH and polyclonal antibodies were generated in goats. Control peptide was used for affinity purification of antibodies (**Cat # HA12-A**).

Aff pure Anti-HA tag IgG was covalently linked agarose (**Cat # HA12-AS**). Antibody concn is approx 0.5 mg/ml of beads. It is supplied as 1:1 suspension of beads in PBS pH 7.4 containing 0.05% azide (0.250 ml agarose beads). Binding capacity of the matrix is approx 100-500 ug HA-tagged protein per ml of the matrix. Binding capacity and elution of fusion tagged proteins will vary depending upon the protein and buffer conditions. Store matrix at 4oC. Do not freeze.

Stability: 6-12 months at -20oC or below.

Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

Anti-tag IgG-Agarose may be used for immunoprecipitation and for affinity purification of fusion proteins containing the tag. The binding capacity of the column for the affinity purification of the fusion protein must also be evaluated for a given fusion protein.

Immunoprecipitation

25-50 ul of gel volume (50-10 ul of 1:1 suspension) per 500 ug of the protein lysate. For recombinant proteins with high level of expression, the lysate amounts must be optimized.

Purification of Ha-tag fusion proteins.

The purification can be performed using a small column or the batch process. Purification can be performed at 4oC (for temp sensitive proteins) or room temp.

1. Apply clear cell lysates to the column and recycle 2-3 times. Cell extracts can be prepared in PBS, pH 7.4 or other suitable buffers.
2. Collect the flow through, and wash until the OD280 is <0.020 or achieved a base line.
3. Elute proteins with 0.1M ammonium hydroxide (pH 11-12) into vials containing 30-50 ul of 1N acetic acid per ml of eluant. Collect 0.5-1 ml fraction (5-10 ml total).
4. Low pH elution (Tris-Gly pH 2.5, followed by neutralization in 1M Tris pH 8.0) can also be used.
5. Affinity column should not be exposed to low or high pH for prolonged periods of time.
6. Binding and elution buffers must be optimized for a given protein.
7. Bound proteins should be dialyzed against PBS or other buffer at 4oC and concentrated if necessary.

Antibody concentration must be optimized for each application under defined experimental conditions.

Stability: 6-12 months at -20oC or below.

Shipping: 4oC for solutions and room temp for powder.

Antibody concentration must be optimized for each application under defined experimental conditions.

General References: Gazin C et al (1984) EMBO J 3, 383-387; Tachibana K et al (1992) Gene, in press.

*This product is for In vitro research use only.

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