

Product Specification Sheet
HA-tag Antibodies and conjugates

Cat. HA13-AS-250

Mouse monoclonal Anti-HA tag IgG-Agarose conjugate

SIZE: 500 ul

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. It is supplied in PBS pH 7.4 at 100 ug/100 ul (1 mg/ml) in solution or powder form.

HA-tag fusion protein was used as immunogen to generate mouse monoclonal. The clone (#HA12-M, isotype IgG2b) was selected for reactivity with HA-tag or fusion protein. Protein A/G purified antibody (**Cat # HA13-M**) is supplied as 1 mg/ml solution in PBS 7.4 at 1 mg/ml solution or as lyophilized powder.

Cat# HA13-AS-250, Agarose conjugate

Aff pure Anti-HA-tag IgG was covalently linked agarose (**Cat # HA13-AS-250**). Antibody concn is approx 1 mg/ml of beads. It is supplied as 1:1 suspension of beads in PBS pH 7.4 containing 0.05% azide (0.50 ml agarose beads). Binding capacity of the matrix is approx ~200 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. The matrix is provided in a small 2 ml spin column. It can be used in the column or used in other tubes or columns.

Stability: 6-12 months at 40C below. Do not Freeze.

Shipping: room temp.

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. It is recommended to use the pre-clarified cell lysate. Binding can be performed in RIPA buffer and eluted with SDS-PAGE sample buffer. Eluted proteins visualized with anti-myc antibody (Cat # HA13-M).

Purification of Ha-tag fusion proteins.

The purification can be performed using a small column or the batch process. Purification can be performed at 40C (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 40C and concentrated if necessary.

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

Control peptide (cat #HA12-P), because of its small size, is not suitable for Western. It can be used to coat ELISA plates, dot blots, or used as antibody blocking peptide (use 10 ug peptide per ul/ug of antibody) to show antibody specificity. Recombinant purified **HA-tag protein control for Western #HA11-C** is available for control studies. **Recombinant purified HA-tag protein #HA15-R** can be used for ELISA etc.

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.

Related items

Antibodies to GST, MBP, Thrx, His, Myc tags and recombinant proteins

HA13-AS-250

80603A

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi - 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com