

## Product Specification Sheet

### Monoclonal Anti-Poly Histidine Tag (His-tag) Antibody & HRP Conjugate

Cat # HISP11-M	Mouse Monoclonal Anti-poly His-Tag, <b>ascites</b>	<b>SIZE:</b> 100 ul
Cat # HISP12-HRP	Mouse Monoclonal Anti-poly His-Tag- <b>HRP Conjugate</b>	<b>SIZE:</b> 100 ul
Cat # HISP13-AP	Mouse Monoclonal Anti-poly His-Tag- <b>AP Conjugate</b>	<b>SIZE:</b> 100 ul
Cat # HISP11-P	Poly His-Tag (His His His His His His)6; or (His)6 control peptide	<b>SIZE:</b> 100 ug

Recombinant DNA technology allows the addition of short pieces of well-defined tags, "peptides" or proteins at the amino or c-terminus of target genes, which can provide 'affinity handles' designed to bind specific matrices. Therefore, tags enables a selective identification and purification of the protein of interest. Poly-His (His x 6) containing proteins can be purified by immobilized metal ion affinity chromatography (IMAC), making use of its high affinity for transition metal ions (1-4). This purification system eliminates the harsh conditions required to elute protein from ligand affinity columns. Numerous recombinant fusion proteins have been engineered with histidine tags to allow detection, isolation and purification of fusion proteins (1-4). Anti-poly may be used to identify the expression of a poly-histidine fusion protein in bacteria, bacterial lysates or cells and tissues transfected with a poly-histidine fusion protein expression vectors.

#### Source of Antibody and Conjugate

Balb/c mice were injected with His-tag-fusion protein and resulting clones were selected to react with poly-his tag. The resulting clone (IgG2a) was expanded as ascites. Purified antibody was coupled to horse radish peroxidase conjugate (HRP) or Alkaline phosphatase (AP) using glutaraldehyde procedure. The conjugate has been purified to remove free enzyme and antibody.

Poly (His)6 (His-tag) peptide was synthesized and purified (>90%) (cat# **HISP11-P**; mol wt 840.87 daltons). It can be used as blocking peptide for all anti-poly his antibodies or to coat the ELISA plates or used for the elution of antibodies.

#### Form & Storage

**Mouse monoclonal antibody, HRP and AP-conjugates** are supplied as 100 ul soln (1 mg/ml) or lyophilized preserved in 0.1% sodium azide.

**Lyophilized products** should be reconstituted in 100 ul water and gently mixed for 15 min at room temp. All peptide/antibody received in solution or reconstituted from Lyophilized vials should be stored frozen at -20°C or below in suitable aliquots. It is not recommended to store diluted solutions. Avoid repeated freeze and thaw.

#### Recommended Usage

Western Blotting (1:1K-5K using Chemiluminescence technique). Antibodies react with native and denatured his-tag containing proteins. Anti-His-AP or -HRP conjugates can be used directly at an appropriate dilution (1-10 K) depending upon the sensitivity of the assay.

**ELISA** (1:10-50K; using 50-100 ng control antigen/well).

**Histochemistry & Immunofluorescence:** We recommend the use of affinity pure antibody at 10-50 ug/ml or enzyme conjugates at 1:200-1:2K.

#### Specificity & Cross-reactivity

Monoclonal Anti-poly-Histidine recognizes native and denatured-reduced forms of synthetic poly-histidine or poly-histidine-tagged fusion proteins in immunoblotting, dot blot and ELISA. The antibodies react with fusion protein expressed by prokaryotic pET, pRSET and pTrc expression vectors.

#### General References:

Narayanan, S., J. Chromatogr., 658, 237 (1994), Casey, J., et al., J. Immunol. Meth., 179, 105 (1995), Uhlen, M., and Moks, T., Meth. Enzymol., 185, 129 (1990), Skerra, A., et al., Bio/Technology, 9, 273 (1991).

\*This product is for In vitro research use only.

#### Other Fusion tag antibodies available from ADI

Recombinant protein with His-tag also available.

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

HISP11-M, HISP-12-HRP, HISP-3-AP 90609A

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