### 6x-His Tag Monoclonal Antibody (3D5)

**Catalog Number:** R930-25

<table>
<thead>
<tr>
<th>Details</th>
<th>Species Reactivity</th>
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</thead>
<tbody>
<tr>
<td><strong>Size</strong></td>
<td>Tested species reactivity</td>
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<tr>
<td>50 µl</td>
<td>Published species reactivity</td>
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<tr>
<td><strong>Host/Isotope</strong></td>
<td>Tag</td>
</tr>
<tr>
<td>Mouse / IgG2b</td>
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<tr>
<td><strong>Class</strong></td>
<td>Tested Applications</td>
</tr>
<tr>
<td>Monoclonal</td>
<td>Dilution *</td>
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<tr>
<td><strong>Type</strong></td>
<td>ELISA (ELISA)</td>
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<tr>
<td>Antibody</td>
<td>Assay Dependent</td>
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<tr>
<td><strong>Clone</strong></td>
<td>Flow Cytometry (Flow)</td>
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<tr>
<td>3D5</td>
<td>1:100-1:500</td>
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<tr>
<td><strong>Immunogen</strong></td>
<td>Immunocytochemistry (ICC)</td>
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<td>6x His synthetic peptide</td>
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<tr>
<td><strong>Conjugate</strong></td>
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<td>Unconjugated</td>
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<td><strong>Form</strong></td>
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<td>Liquid</td>
<td>Western Blot (WB)</td>
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<td><strong>Purification</strong></td>
<td>1:1000-1:5000</td>
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<td>purified</td>
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<td><strong>Storage buffer</strong></td>
<td>Western Blot (WB)</td>
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<tr>
<td>PBS</td>
<td>See 5 publications below</td>
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<tr>
<td><strong>Contains</strong></td>
<td>Miscellaneous PubMed (MISC)</td>
</tr>
<tr>
<td>0.01% sodium azide</td>
<td>See 3 publications below</td>
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<tr>
<td><strong>Storage Conditions</strong></td>
<td>Immunocytochemistry (ICC)</td>
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<tr>
<td>Store at 4°C short term.</td>
<td>See 1 publications below</td>
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<tr>
<td>For long term storage,</td>
<td>Immunoprecipitation (IP)</td>
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<tr>
<td>store at -20°C, avoiding</td>
<td>See 1 publications below</td>
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<tr>
<td>freeze/thaw cycles.</td>
<td><strong>Suggested working dilutions</strong></td>
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<tr>
<td></td>
<td>are given as a guide only.</td>
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<tr>
<td></td>
<td>It is recommended that the</td>
</tr>
<tr>
<td></td>
<td>user titrate the product</td>
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<tr>
<td></td>
<td>for use in their own experiment using appropriate negative and positive controls.</td>
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</tbody>
</table>

### Product specific information

NOTE: R930-25 was previously sold under product #46-0693. When re-ordering, please use #R930-25.

This antibody can be used to detect expression of C-terminal His fusion proteins from bacterial, insect, and mammalian cells.

This product contains enough material for 25 Western blots. It is functionally tested using 500ng of an E. coli expressed fusion protein containing a C-terminal polyhistidine (6xHis) epitope. A signal can be detected when tested against 100ng of recombinant Positope™ protein. For Western blot, dilute in PBS containing 0.05% Tween-20 and 5% nonfat, dry milk (PBSTM).

This product has also been used successfully to immunoprecipitate fusion proteins that contain the 6x His tag.

Using chemiluminescence as the detection method, no cross-reactivity has been observed in bacterial lysates. In mammalian lysates, a few cross-reactive proteins have been observed upon overexposure of blots.

### Background/Target Information

The His tag is a synthetic oligo peptide consisting of 6 consecutive histidine residues (HHHHHH). It is commonly expressed as a tag at either N- or C-terminal regions of recombinant proteins to allow isolation or purification by immobilized metal affinity chromatography. The affinity of the histidine-tag motif to Ni²⁺ by chelation is strong and selective enough to enable purification of the protein to homogeneity by affinity chromatography on a Ni²⁺-NTA adsorbent. His tag-specific antibodies are used to facilitate detection or coimmunoprecipitation of tagged proteins.

6x-His Tag Antibody (R930-25) in IF

Immunofluorescence analysis of Histidine tag (6XHis) was done on HEK-293 cells transiently overexpressing His-IGFBP6. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton X-100 for 10 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with Histidine (C-term) tag (6XHis) Mouse Monoclonal Antibody (Product # R930-25) at a dilution of 1:400 in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor® 488 Rabbit Anti-Mouse IgG Secondary Antibody (Product # A-11059) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor® 594 Phalloidin (Product # A12381). Panel d is a merged image showing cytoplasmic localization. Panel e is untransfected HEK-293 cells. The images were captured using a Nikon microscope at 20X magnification.

6x-His Tag Antibody (R930-25) in WB

Western blot analysis of Histidine tag (6XHis) was performed by loading 20 µg of whole cell extracts of untransfected HEK-293 and HEK-293 transiently overexpressing His-IGFBP6 using Novex® NuPAGE® 4-12% Bis-Tris gel (Product # NP0321BOX), Xcell SureLock™ system (Product # EI0002), Novex sharp Pre-stained Protein Standard (LC5800), and iBlot® Dry Blotting System (IB21001). Proteins were transferred to a nitrocellulose membrane and blocked with 5% skim milk for at least 1 hour at room temperature. His-IGFBP6 was detected at ~31 kDa using Histidine (C-term) tag (6XHis) Mouse Monoclonal Antibody (Product # R930-25) at 1:1000 dilution in 2.5% skim milk at 4°C overnight on a rocking platform. Goat Anti-Mouse IgG - HRP Secondary Antibody (Product # 62-6520) at a 1:4000 dilution was used and chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (WP20005).
**5 Western Blot References**

**Species / Dilution**

**Summary**

- **R930-25** was used in western blot to study the increase in translational yields by recruiting mRNA as measured by profiling active ribosomes by synthetic chimeras with orthogonal ribosomal proteins.

Not Applicable / 1:5000

- Biotechnologoy progress (Mar 2016; 32: 285)

  **"Synthetic chimeras with orthogonal ribosomal proteins increase translation yields by recruiting mRNA for translation as measured by profiling active ribosomes."**

  **Author(s): Cho SH, Contreras LM, Ju SH**

  PubMed Article URL: http://dx.doi.org/10.1002/btpr.2227

- **R930-25** was used in western blot to identify epitopes of Asp v 13.

Not Applicable / Not Cited

- Molecular immunology (Sep 2011; 48: 1827)

  **"Characterization of the 41kDa allergen Asp v 13, a subtilisin-like serine protease from Aspergillus versicolor."**

  **Author(s): Shi C, Miller JD**

  PubMed Article URL: http://dx.doi.org/10.1016/j.molimm.2011.05.010

- **R930-25** was used in western blot to investigate the mechanism by which AKAP15 targets PKA to L-type Ca(2+) channels.

Not Applicable / 1:1000

- The Journal of biological chemistry (Feb 2002; 277: 4079)

  **"A novel leucine zipper targets AKAP15 and cyclic AMP-dependent protein kinase to the C terminus of the skeletal muscle Ca2+ channel and modulates its function."**

  **Author(s): Hulme JT, Ahn M, Hauschka SD, Scheuer T, Catterall WA**

  PubMed Article URL: http://dx.doi.org/10.1074/jbc.M109814200

- **R930-25** was used in western blot to study regulation of TorC in E. coli.

Not Applicable / Not Cited

- Proceedings of the National Academy of Sciences of the United States of America (Sep 2001; 98: 11615)

  **"An unsuspected autoregulatory pathway involving apocytochrome TorC and sensor TorS in Escherichia coli."**

  **Author(s): Gon S, Jourlin-Castelli C, Théraulaz L, Méjean V**

  PubMed Article URL: http://dx.doi.org/10.1073/pnas.211330598

- **R930-25** was used in western blot to study the regulation of beta-lactamase and penicillin-binding protein 2a.

Not Applicable / Not Cited


  **"A proteolytic transmembrane signaling pathway and resistance to beta-lactams in staphylococci."**

  **Author(s): Zhang HZ, Hackbarth CJ, Chansky KM, Chambers HF**

  PubMed Article URL: http://dx.doi.org/10.1126/science.1055144

**3 Miscellaneous PubMed References**

**Species / Dilution**

**Summary**

- **R930-25** was used in western blot to study optimization of a universal flu vaccine.

Not Applicable / Not Cited

- Human vaccines and immunotherapeutics (Sep 2014; 10: 1211)

  **"Intranasal immunization with influenza antigens conjugated with cholera toxin subunit B stimulates broad spectrum immunity against influenza viruses."**

  **Author(s): Li J, Arévalo MT, Chen Y, Posadas O, Smith JA, Zeng M**

  PubMed Article URL: http://dx.doi.org/10.1074/jimmunol.130032184

- **R930-25** was used in western blot to investigate the roles of gap junctions in neuronal circuitry.

Not Applicable / Not Cited

- The European journal of neuroscience (Jan 2012; 35: 166)

  **"The effector and scaffolding proteins AF6 and MUPP1 interact with connexin36 and localize at gap junctions that form electrical synapses in rodent brain."**

  **Author(s): Li X, Lynn BD, Nagy JI**

  PubMed Article URL: http://dx.doi.org/10.1111/j.1460-9568.2011.07947.x

- **R930-25** was used in western blot to investigate Pannexin1 or Pannexin2 complex.

Not Applicable / Not Cited


  **"Pannexin1 and Pannexin2 channels show quaternary similarities to connexons and different oligomerization numbers from each other."**


  PubMed Article URL: http://dx.doi.org/10.1074/jbc.M110.115444

**1 Immunocytochemistry References**

**Species / Dilution**

**Summary**


Products are warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and accompanying package inserts (“Documentation”). Its claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.

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<thead>
<tr>
<th>Species / Dilution</th>
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<tr>
<td>R930-25 was used in immunoprecipitation to study the role of FEZL-SEMA5A pathway in neuronal development and innate immunity in cows.</td>
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<tr>
<td>R930-25 was used in immunocytochemistry to describe methods to examine nuclear pore complexes using Xenopus oocytes.</td>
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