

Anti-HES-1

Polyclonal Antibody

Cat. # AB5702

Lot # LV1544120

pack size: 100 µg

Store at -70°C

FOR RESEARCH USE ONLY



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IH	H, M	N/A	N/A	Rb	29 kDa	NP_005515

Background

Hes1 is a transcriptional repressor of genes that require a bHLH protein for their transcription. It may act as a negative regulator of myogenesis by inhibiting the functions of MYOD1 and ASH1. Hes1 is expressed in developing neuroectodermal and endodermal endocrine tissues, and Hes1 deficient embryos show severe defects in neuronal development, as well as pancreatic hypoplasia.

Presentation

Purified rabbit polyclonal in buffer containing a solution of 50% saturated ammonium sulfate and PBS containing no preservatives.

Specificity

Recognizes HES-1 (Hairy family), a Helix-loop-helix class of transcription factor.

Species Cross-reactivity

Human and rodent. Reactivity with other species has not been confirmed.

Immunogen

Synthetic peptide.

Molecular Weight

29 kDa

Method of Purification

Immunoaffinity Purified

Storage and Handling

Stable for 6 months unopened at -70°C from date of receipt. Avoid repeated freeze/thaw cycles.

The rehydrated antibody solutions can be stored undiluted at 2-8°C for 2 months without any significant loss of activity. Note, the solution is not sterile, thus care should be taken if product is stored at 2-8°C.

For storage at -20°C, the addition of an equal volume of glycerol can be used, however, it is recommended that ACS grade or higher glycerol be used, as significant loss of activity can occur if the glycerol used is not of high quality.

For freezing, it is recommended that the rehydrated antibody solution be further diluted 1:1 with a 2% BSA (fraction V, highest-grade available) solution made with the rehydration buffer. The resulting 1% BSA/antibody solution can be aliquoted and stored frozen at -70°C for up to 6 months.

Control

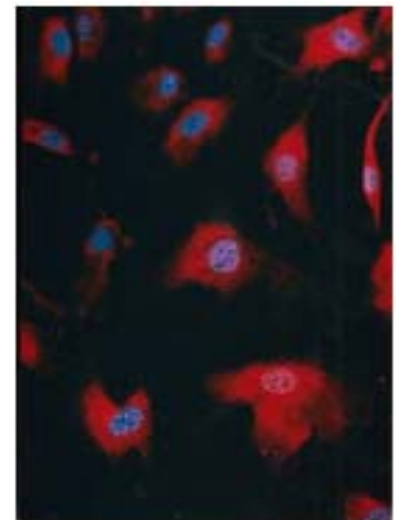
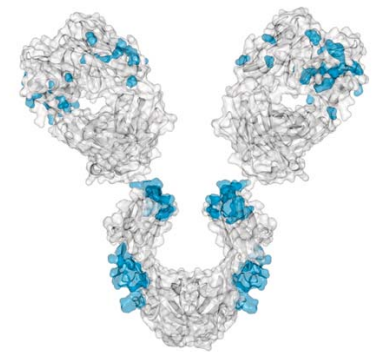
Human neural stem cells, Raji cell lysate.

Research Applications

Western Blot: 1:200-1:1,000 dilution using ECL.

Immunohistochemistry: 1:200-1:1,000 dilution of a previous lot worked.

Optimal working dilutions must be determined by the end user.



Immunohistochemistry: Representative lot data. Rabbit anti-HES-1 (Catalog Number AB5702) immunofluorescence staining of paraformaldehyde-fixed differentiated human neural stem cells showing spotted nuclear and cytoplasmic localization of HES-1.

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence
IH Immunohistochemistry (Tissue)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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PREPARATION AND USE

To reconstitute the antibody, centrifuge the antibody vial at moderate speed (5,000 rpm) for 5 minutes to pellet the precipitated antibody product. Carefully remove the ammonium sulfate/PBS buffer solution and discard. It is not necessary to remove all of the ammonium sulfate/PBS solution: 10 µL of residual ammonium sulfate solution will not effect the resuspension of the antibody. Do not let the protein pellet dry, as severe loss of antibody reactivity can occur.

Resuspend the antibody pellet in any suitable biological buffer, standard PBS or TBS (pH 7.3-7.5) are typical. Volumes required are not critical but it is suggested that the final antibody concentration be between 0.1 mg/mL and 1.0 mg/mL. For example, to achieve a 1 mg/mL concentration with 50 µg of precipitated antibody, the amount of buffer needed would be 50 µL.

Carefully add the liquid buffer to the pellet. DO NOT VORTEX. Mix by gentle stirring with a wide pipet tip or gentle finger-tapping. Let the precipitated antibody rehydrate for 1 hour at 4-25C° prior to use. Small particles of precipitated antibody that fail to resuspend are normal. Vials are overfilled to compensate for any losses.

Important Note: During shipment, small volumes of antibody will occasionally become entrapped in the seal of the product vial. For antibodies with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

PROTOCOL

Immunohistochemistry

1. Dissect tissues and freeze on dry ice
2. Cut on a cryostat – 10 um slices
3. Dry slides at room temperature for 30-90 min
4. Fix with cold Acetone/Methanol (50/50) 2 min - then either process further or store at -20°C. Alternative is fixation with 4% PFA and treatment with a trypsin solution. (0.05% trypsin , incubation 10 minutes at 37°C, followed by 3 washes with PBS)
5. Let air dry
6. PBS 5min
7. Incubate in 50 mM ammonium chloride 30 min
8. PBS 5min
9. Blocking serum 30-45min
10. Primary antibody 90 min - dilution 1:100 - 1:600
11. Wash 3 times for 5 min
12. Secondary fluorescent conjug. antibody 30min
13. Wash 3 times for 5 min and mount

RELATED PRODUCTS (specific)

cat #	description
AB15470	■ Anti-HES-1
AB5702	■ Anti-HES-1
AB5704	■ Anti-HES-2
AB5965	■ Anti-HES-4
AB5708	■ Anti-HES-5

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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