

Product Datasheet

Lactate Dehydrogenase A/LDHA Antibody - BSA Free NBP1-48336

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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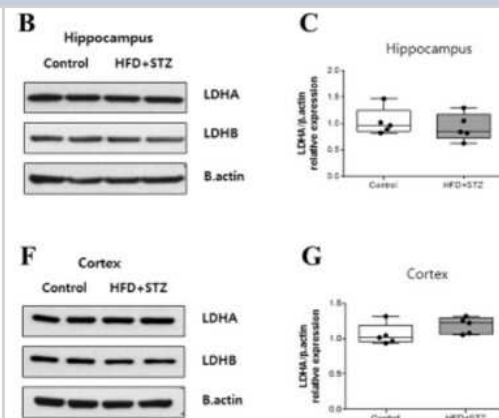
NBP1-48336**Lactate Dehydrogenase A/LDHA Antibody - BSA Free**

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	36 kDa
Product Description	
Host	Rabbit
Gene ID	3939
Gene Symbol	LDHA
Species	Human, Mouse, Porcine, Bovine
Reactivity Notes	Porcine reactivity reported in scientific literature (Elgin et al).
Immunogen	Synthetic peptide made to a C-terminal portion of the human Lactate Dehydrogenase A protein (within residues 280-332). [Swiss-Prot# P00338]
Product Application Details	
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 0.5ug/ml, Simple Western 1:50, Flow Cytometry, ELISA reported in scientific literature (PMID 35052722), Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry-Paraffin 1:100, Immunohistochemistry-Frozen reported in scientific literature (PMID 25004202)
Application Notes	<p>In Western blot, a band is seen at approx. 36 kDa. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.1 mg/mL, separated by Size, antibody dilution of 1:50, apparent MW was 40 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

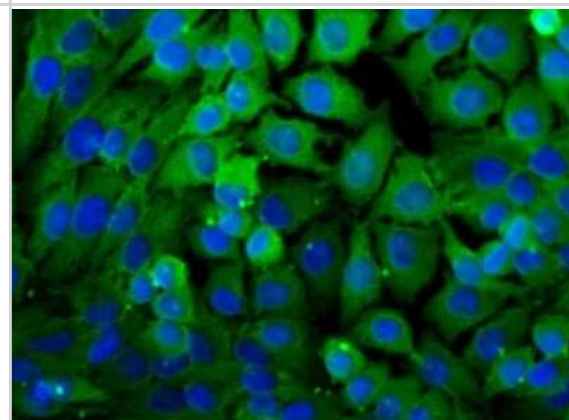


Images

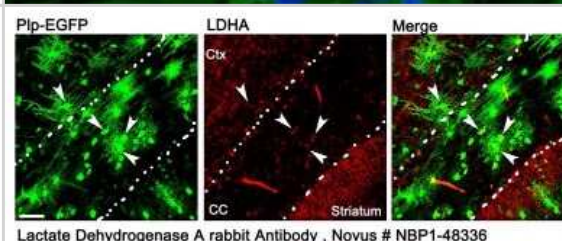
Western Blot: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Increased lactate level detected in the hippocampus of streptozotocin-injected mice fed an HFD (HFD + STZ).b LDHA and LDHB protein levels were measured by western blot assay. c LDHA protein levels were quantified by the ratio to B-actin in the hippocampus. f Cropped images of LDHA and LDHB in the cortex. g Quantified LDHA in the cortex by the ratio to B-actin. Values are expressed as means \pm SEMs (n = 5 for both groups). * p < 0.05. Image collected and cropped by Citeab from the following publication (Hyperpolarized [^{13}C]lactate flux increased in the hippocampal region in diabetic mice. Mol Brain (2019) licensed under a CC-BY license.



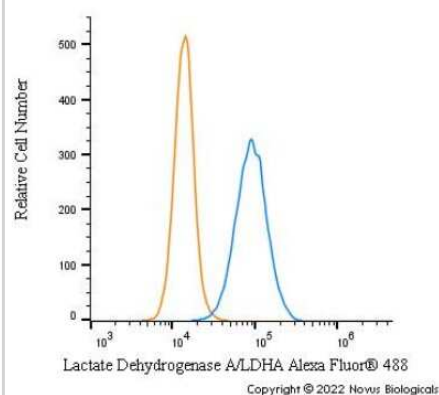
Immunocytochemistry/Immunofluorescence: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Immunocytochemical analysis of Lactate Dehydrogenase A in HeLa cells



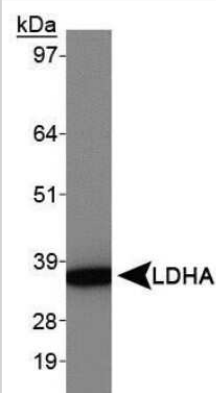
Immunohistochemistry: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Staining of postnatal day 8(P8) Plp-EGFP transgenic mice brain section. Image from verified customer review.



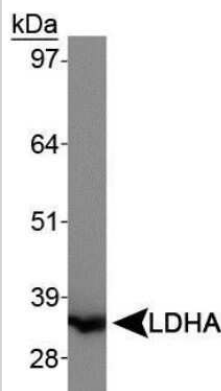
Flow Cytometry: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - An intracellular stain was performed on HeLa cells with Lactate Dehydrogenase A/LDHA Antibody NBP1-48336AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 $\mu\text{g}/\text{mL}$ for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



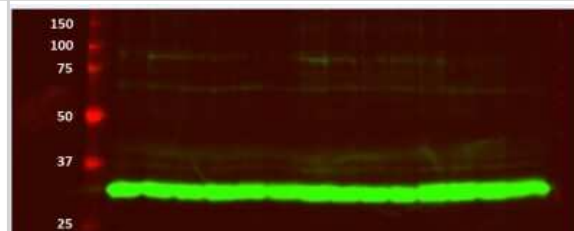
Western Blot: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Analysis of Lactate Dehydrogenase A in HeLa whole cell lysates.



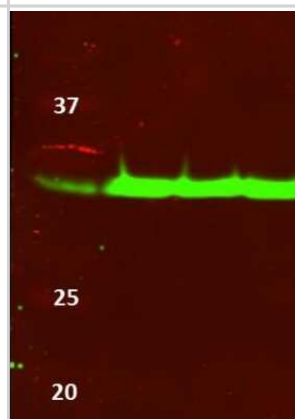
Western Blot: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Analysis of Lactate Dehydrogenase A in NIH/3T3 whole cell lysates.



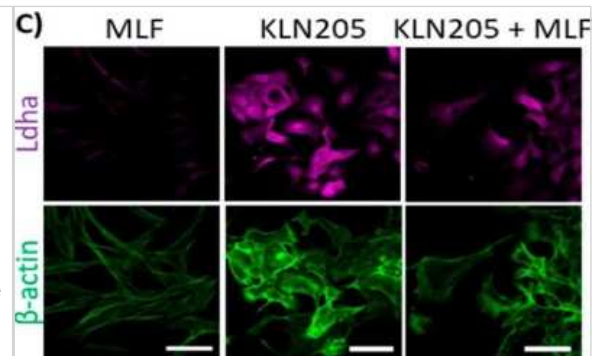
Western Blot: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Lactate Dehydrogenase A expression in bovine skeletal muscle lysate. Image from verified customer review.



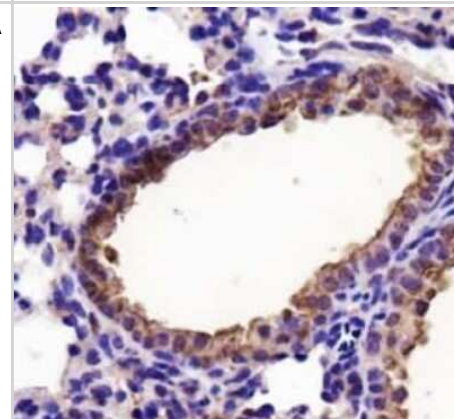
Western Blot: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Lactate Dehydrogenase A expression in porcine skeletal muscle lysate. Image from verified customer review.



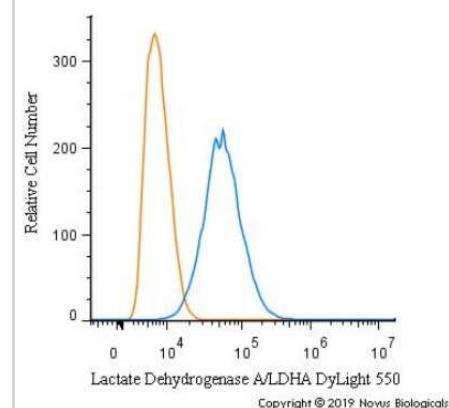
Immunocytochemistry/Immunofluorescence: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - The effect of the co-culturing of cancer cells and fibroblasts on the expression of Hk2 and Ldha. In the co-cultures, the expression of Ldha was reduced in cancer cells, but was elevated in fibroblasts. Bar = 50 um. The ratio of the fluorescence of Hk2 and Ldha to beta-actin in the MLF monoculture was assumed to be 1. All the experiments were performed in triplicate and obtained similar results, and representative data from one experiment are shown in the figure. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31947613/>) licensed under a CC-BY license.



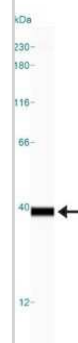
Immunohistochemistry: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Staining of Lactate Dehydrogenase A in lung tissue.



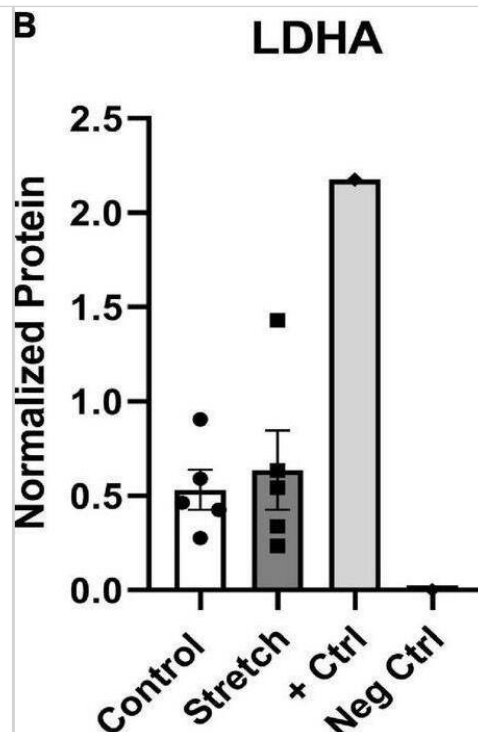
Flow Cytometry: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336]



Simple Western: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Lane view shows a specific band for Lactate dehydrogenase A in 0.1 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230kDa separation system.



Simple Western: Lactate Dehydrogenase A/LDHA Antibody - BSA Free [NBP1-48336] - Protein changes in ONHAs corroborate bioenergetics data. (A) Glucose transporter-1 protein levels in Stretched ONH astrocytes are significantly higher than Control (* $p = 0.0225$, $n = 7$ Control, $n = 8$ Stretch). Retinal lysate from a 2 month-old mouse was used as a positive control for each protein analyzed, while negative control was the signal obtained when no primary antibody was included in the capillary. (B) Lactate dehydrogenase-A, the astrocyte-specific isoform of the enzyme that catalyzes the interconversion of pyruvate & lactate, has equivalent protein levels in Control & Stretch ONH astrocytes. (C) Glucose-6-phosphate dehydrogenase, the enzyme that shunts glucose into the pentose phosphate pathway, is no different in Control & Stretch ONH astrocytes. (D) Glutamine synthetase, the enzyme that synthesizes glutamine from glutamate, is no different in Control & Stretch ONH astrocytes. (E) The monomeric form of glutamate-aspartate transporter (GLAST) has significantly higher protein levels in the Stretch as compared to the Control ONH astrocytes ($p = 0.020$; $n = 4$ Control, $n = 5$ Stretch). (F) GLAST dimer protein levels are no different in Control & Stretch ONH astrocytes. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35992925>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Abigail M Maucieri, David H Townson Evaluating the impact of the hexosamine biosynthesis pathway and O-GlcNAcylation on glucose metabolism in bovine granulosa cells. Molecular and cellular endocrinology 2023-02-28 [PMID: 36690170]

Matsumoto S, Kishimoto S, Saito K et al. Metabolic and physiologic imaging biomarkers of the tumor microenvironment predict treatment outcome with radiation or a hypoxia-activated prodrug in mice Cancer Res. 2018-05-23 [PMID: 29792309]

Jason D Yang, Daniel Mott, Rujapak Sutiwisesak, Yu-Jung Lu, Fiona Raso, Britni Stowell, Greg Hunter Babunovic, Jinhee Lee, Steve M Carpenter, Sing Sing Way, Sarah M Fortune, Samuel M Behar Mycobacterium tuberculosis-specific CD4+ and CD8+ T cells differ in their capacity to recognize infected macrophages. PLoS pathogens 2018-07-06 [PMID: 29782535]

Melissa Schwab, Ali Bashiri Dezfouli, Mohammad Khosravi, Bayan Alkotub, Lisa Bauer, Mohammad Javed Tahmasebi Birgani, Gabriele Multhoff The radiation- and chemo-sensitizing capacity of diclofenac can be predicted by a decreased lactate metabolism and stress response. Radiation oncology (London, England) 2024-01-18 [PMID: 38229111]

Ahmad F, White M, Yamamoto K et al. Metabolic and imaging phenotypes associated with RB1 loss in castrate resistant prostate cancer bioRxiv 2023-11-17 (WB, Human)

Wicks JC Understanding Beef Quality Development and Different Feeding Regimes Thesis 2023-01-01 (WB, Bovine)

Details:
1:30000 dilution

Nsiah NY, Inman DM. Destabilizing COXIV in Müller Glia Increases Retinal Glycolysis and Alters Scotopic Electroretinogram Cells 2022-11-24 [PMID: 36497016] (Immunocytochemistry/ Immunofluorescence)

Gizak A, McCubrey JA, Rakus D. Cell-to-cell lactate shuttle operates in heart and is important in age-related heart failure Aging (Albany NY) 2020-02-08 [PMID: 32035422]

Schwab M, Thunborg K, Azimzadeh O et al. Targeting Cancer Metabolism Breaks Radioresistance by Impairing the Stress Response Cancers (Basel) 2021-07-27 [PMID: 34359663] (Western Blot, Block/Neutralize)

Rimmer L, Geisbrecht E, Chao M et al. Investigating the metabolic changes that accompany skeletal muscle maturation Physiology 2023-05-01

Schwab M, Dezfouli A, Khosravi M et al. The radiation- and chemo-sensitizing capacity of diclofenac can be predicted by a decreased lactate metabolism and stress response Research Square 2023-03-20 (WB)

Rimmer L Investigating the metabolic changes that accompany skeletal muscle hypertrophy Thesis 2023-01-01 (Western Blot)

More publications at <http://www.novusbio.com/NBP1-48336>

Procedures

Western Blot Protocol for Lactate Dehydrogenase A/LDHA Antibody (NBP1-48336)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunocytochemistry/ Immunofluorescence Protocol for Lactate Dehydrogenase A/LDHA Antibody (NBP1-48336)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.



Immunohistochemistry-Paraffin Protocol for Lactate Dehydrogenase A/LDHA Antibody (NBP1-48336)**Immunohistochemistry-Paraffin Embedded Sections****Antigen Unmasking:**

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.



Flow (Intracellular) Protocol for Lactate Dehydrogenase A/LDHA Antibody (NBP1-48336)**Protocol for Flow Cytometry Intracellular Staining****Sample Preparation.**

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 μ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
 - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 1 mL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

Optional: Perform cell surface staining as described in the previous section.

1. Fix the cells by adding 100 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
 - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
 - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 μ L/ 1×10^6 cells of primary antibody or 1-3 μ L/ 1×10^6 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer + 0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 μ L per sample and proceed with flow analysis.



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Products Related to NBP1-48336

NB800-PC1	HeLa Whole Cell Lysate
NBP1-48336PEP	Lactate Dehydrogenase A/LDHA Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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