Anti-Alpha B Crystallin Antibody [JA50-32]

ET1704-60

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Rat
Applications:	WB, IHC-P, IF-Cell, IF-Tissue, IP, FC
Molecular Wt:	Predicted band size: 20 kDa
Clone number:	JA50-32

Description: Crystallins are the major proteins of the vertebrate eye lens, where they maintain the transparency and refractive index of the lens. Crystallins are divided into α, β and γ families, and the β- and γ-crystallins also compose a superfamily. Crystallins usually contain seven distinct protein regions, inclu-ding four homologous motifs, a connecting peptide, and N- and C-terminal extensions. α-crystallins consist of three gene products, αA-, αB- and αC-crystallin, which are members of the small heat shock protein family (HSP 20). α-crystallins act as molecular chaperones by holding denatured proteins in large soluble aggregates. However, unlike other molecular chaperones, α-crystallins do not renature these proteins. Expression of αA-crystallin is restricted to the lens and defects of this gene cause the development of autosomal dominant congenital cataracts (ADCC). The human αB-crystallin gene product is expressed in many tissues, including lens, heart and skeletal muscle. Elevated expression of αB-crystallin is associated with many neurological diseases, and a missense mutation in this gene has co-segregated in a family with a Desmin-related myopathy.

Immunogen: Synthetic peptide within Human Alpha B Crystallin aa 126-175 / 175.

Positive control: U-2 OS cell lysate, rat heart tissue lysate, human skeletal muscle tissue lysate, Hela, human embryonic skeletal muscle tissue, human prostate tissue, human cardiac muscle tissue, rat cardiac muscle tissue, U-2 OS.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: P02511 Human | P23928 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

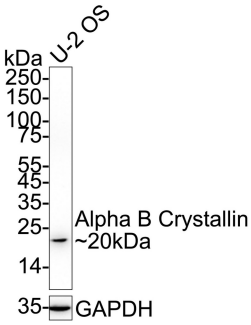

Fig1: Western blot analysis of Alpha B Crystallin on different lysates with Rabbit anti-Alpha B Crystallin antibody (ET1704-60) at 1/1,000 dilution.

Lane 1: U-2 OS cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 20 kDa
Observed band size: 20 kDa

Exposure time: 59 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1704-60) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

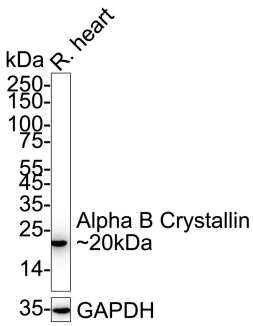

Fig2: Western blot analysis of Alpha B Crystallin on different lysates with Rabbit anti-Alpha B Crystallin antibody (ET1704-60) at 1/1,000 dilution.

Lane 1: Rat heart tissue lysate

Lysates/proteins at 40 µg/Lane.

Predicted band size: 20 kDa
Observed band size: 20 kDa

Exposure time: 2 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1704-60) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

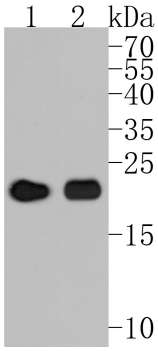

Fig3: Western blot analysis of Alpha B Crystallin on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1704-60, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:20,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Rat heart tissue lysate
Lane 2: Human skeletal muscle tissue lysate

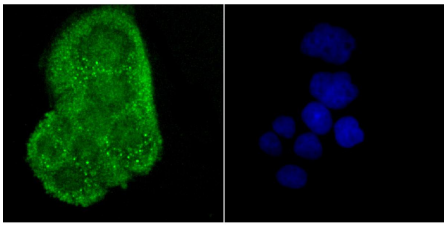

Fig4: ICC staining of Alpha B Crystallin in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-60, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

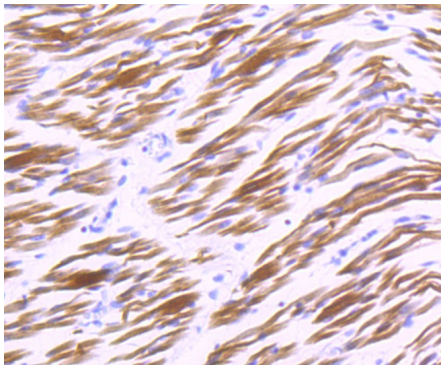

Fig5: Immunohistochemical analysis of paraffin-embedded human embryonic skeletal muscle tissue using anti-Alpha B Crystallin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-60, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

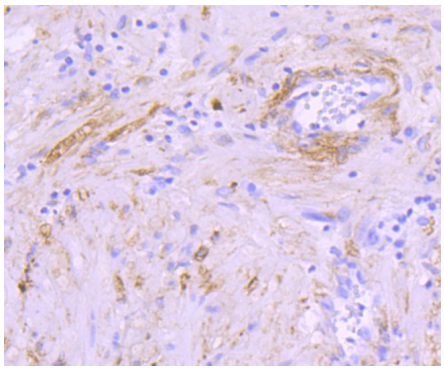

Fig6: Immunohistochemical analysis of paraffin-embedded human prostate tissue using anti-Alpha B Crystallin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-60, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

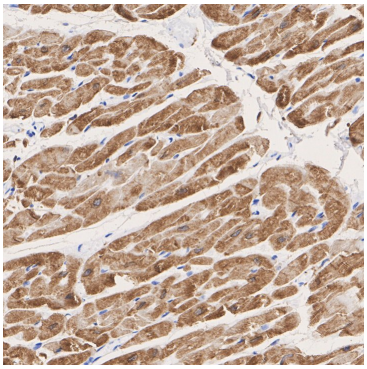

Fig7: Immunohistochemical analysis of paraffin-embedded human cardiac muscle tissue with Rabbit anti-Alpha B Crystallin antibody (ET1704-60) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-60) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

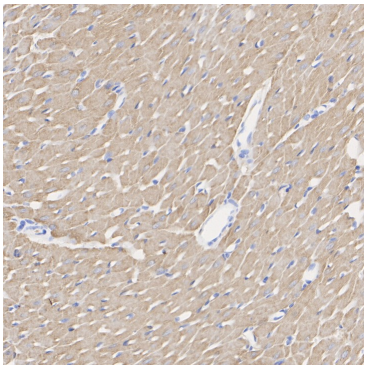

Fig8: Immunohistochemical analysis of paraffin-embedded rat cardiac muscle tissue with Rabbit anti-Alpha B Crystallin antibody (ET1704-60) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1704-60) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

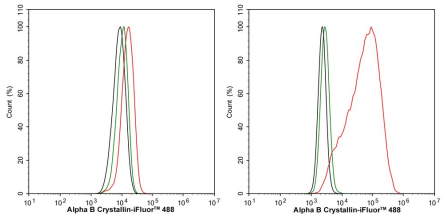

Fig9: Flow cytometric analysis of MCF7 (left, negative) and U-2 OS (right, positive) cells labeling Alpha B Crystallin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1704-60, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Sosunov, A.A. et al. Phenotypic conversions of "protoplasmic" to "reactive" astrocytes in Alexander disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 33: 7439-50 (2013).
2. Lambrecht S et al. Differential expression of alphaB-crystallin and evidence of its role as a mediator of matrix gene expression in osteoarthritis. Arthritis Rheum 60:179-88 (2009).