# abcam

#### Product datasheet

## Anti-Grp75/MOT antibody [JG1] ab2799

★★★★★ <u>14 Abreviews</u> <u>51 References</u> 10 Images

Overview

Product name Anti-Grp75/MOT antibody [JG1]

**Description** Mouse monoclonal [JG1] to Grp75/MOT

Host species Mouse

**Tested applications** Suitable for: ICC/IF, IP, IHC-P, Flow Cyt, WB

Species reactivity Reacts with: Mouse, Rat, Guinea pig, Hamster, Dog, Human, Non human primates

Predicted to work with: Cow

**Immunogen** Synthetic peptide corresponding to Mouse Grp75/MOT aa 661-679.

Sequence:

GSGSSGTGEQKEDQKEEKQ

Database link: P38647

Run BLAST with
Run BLAST with

**Positive control** Recombinant Human Grp75/MOT protein (<u>ab79145</u>) can be used as a positive control in WB.

IHC-P: Human testis tissue. ICC/IF: A549, HMVEC, NS-1, P19, HeLa and human fibroblasts cells.

General notes

The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or

contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

**Properties** 

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.05% Sodium azide

Constituents: PBS, BSA

Purity Protein A purified

Primary antibody notes The HSP 70 family is a set of highly conserved proteins that are induced by a variety of biological

1

stresses, including heat stress, in every organism in which the proteins have been examined. The human HSP 70 family members include: HSP 70, a 70 kDa protein which is strongly inducible in all organisms but which is also constitutively expressed in primate cells; HSP 72, a 72 kDa protein which is induced exclusively under stress conditions; HSC 70, or cognate protein, is a 72 kDa constitutively expressed protein which is involved in uncoating clathrin-coated vesicles; GRP 78, or BiP, is a glucose-regulated 78 kDa protein localized to the endoplasmic reticulum; and mitochondrial HSP 70 (mtHSP 70, GRP 75 or mortalin) a 75 kDa protein that is found within the mitochondria. mtHSP 70 is a mitochondrial resident protein that is involved in protein translocation into the mitochondria. Preproteins cross the mitochondrial membranes in an extended conformation. This requires unfolding of preproteins before entering translocation pores in the mitochondrial outer membrane. Preprotein unfolding is thought to be mediated by mtHSP 70 and other inner membrane proteins of the mitochondria.

**Clonality** Monoclonal

Clone number JG1

Isotype IgG3

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab2799 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews         | Notes  |
|-------------|-------------------|--|
| ICC/IF      | ★★★★ (3)          | 1/50 - 1/200. Staining of mtHSP 70 in DAP.3 cells results in a worm-like staining pattern, consistent with mitochondrial localization. |
| IP          |                   | Use at an assay dependent concentration.   |
| IHC-P       | <b>★★★★</b> (1)   | Use a concentration of 1 µg/ml.  |
| Flow Cyt    |                   | 1/100.  ab91537 - Mouse monoclonal lgG3, is suitable for use as an isotype control with this antibody.                                 |
| WB          | <b>★★★★★ (10)</b> | Use at an assay dependent concentration.   |

#### **Target**

**Function** Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone.

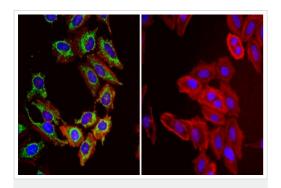
**Sequence similarities** Belongs to the heat shock protein 70 family.

**Post-translational** Phosphorylated upon DNA damage, probably by ATM or ATR.

modifications

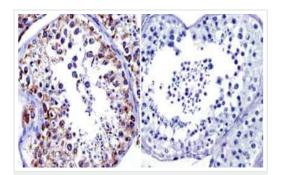
Cellular localization Mitochondrion.

### Images



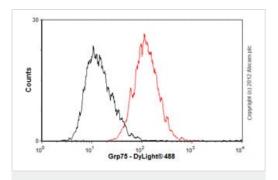
Immunocytochemistry/ Immunofluorescence - Anti-Grp75/MOT antibody [JG1] (ab2799)

Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Grp75/MOT (green) with ab2799. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% BSA for 15 minutes at room temperature. Cells were incubated with (left panel) or without (right panel) ab2799 (1:50) for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat-anti-mouse lgG secondary antibody (1:400) for 30 minutes at room temperature. F-Actin (red) was stained with Dylight 554 phalloidin, and nuclei (blue) were stained with Hoechst 33342 dye. Images were taken at 20X magnification.



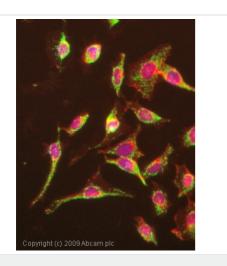
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Grp75/MOT antibody
[JG1] (ab2799)

Immunohistochemistry was performed on normal biopsies of deparaffinized human testis tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer and microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a Mouse Monoclonal Antibody recognizing Grp75/MOT (ab2799) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



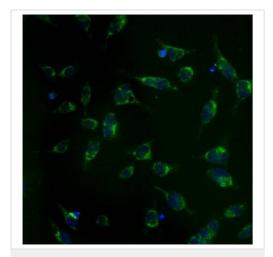
Flow Cytometry - Anti-Grp75/MOT antibody [JG1] (ab2799)

Overlay histogram showing HepG2 cells stained with ab2799 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab2799, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG3 [MG3-35] (ab18394, 1µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed.



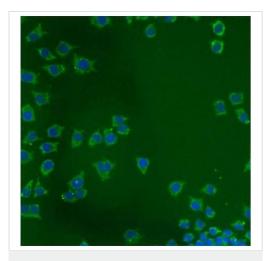
Immunocytochemistry/ Immunofluorescence - Anti-Grp75/MOT antibody [JG1] (ab2799)

ICC/IF image of ab2799 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab2799, 1 $\mu$ g/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse lgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 $\mu$ M.



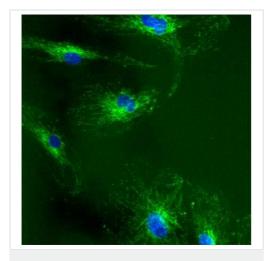
Immunocytochemistry/ Immunofluorescence - Anti-Grp75/MOT antibody [JG1] (ab2799)

Immunocytochemistry/ Immunofluorescence of P19 (Mouse embryonal carcinoma cell line) cells using ab2799.



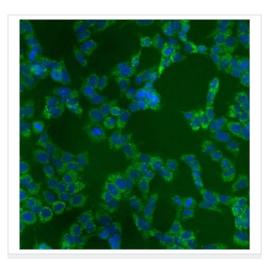
Immunocytochemistry/ Immunofluorescence - Anti-Grp75/MOT antibody [JG1] (ab2799)

Immunocytochemistry/ Immunofluorescence of NS-1 (Mouse myeloma cell line) cells using ab2799.



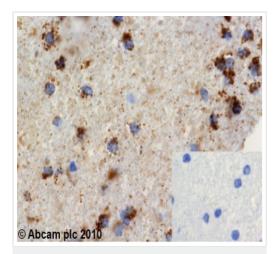
Immunocytochemistry/ Immunofluorescence - Anti-Grp75/MOT antibody [JG1] (ab2799)

Immunocytochemistry/ Immunofluorescence of HMVEC (Human microvascular endothelial cell line) cells using ab2799.



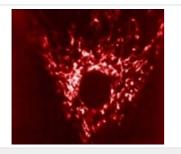
Immunocytochemistry/ Immunofluorescence - Anti-Grp75/MOT antibody [JG1] (ab2799)

Immunocytochemistry/ Immunofluorescence of A549 (Human lung carcinoma cell line) cells using ab2799.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Grp75/MOT antibody
[JG1] (ab2799)

ab2799 (1µg/ml) staining GrP75/MOT in human frontal cerebral cortex using an automated system (DAKO Autostainer Plus). Using this protocol there is strong mitochondrial staining. Insert depicts negative control (no primary antibody). Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



Immunocytochemistry/ Immunofluorescence - Anti-Grp75/MOT antibody [JG1] (ab2799)

Immunocytochemistry/Immunofluorescence analysis of human fibroblasts labelling Grp75/MOT with ab2799.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- · We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

#### Terms and conditions

| • | Guarantee only valid for products bought direct from Abcam or one of our authorized distributors |
|---|--|
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   | 7  |