HSP70 Monoclonal Antibody (3A3)

**Catalog Number** MA3-006

### Details
- **Size**: 50 µL
- **Host/Isotope**: Mouse / IgG1
- **Class**: Monoclonal
- **Type**: Antibody
- **Clone**: 3A3
- **Immunogen**: Human recombinant HSP70 overexpressed in E. coli.

**Conjugate**: Unconjugated

**Form**: Liquid

**Storage buffer**: ascites diluted in PBS

**Contains**: 0.05% sodium azide

**Storage Conditions**: -20° C, Avoid Freeze/Thaw Cycles

### Species Reactivity
- **Tested species reactivity**: Amphibian, Arthropod, Avian, Fruit fly, Fish, Human, Mollusc, Mouse, Non-human primate, Plant, Pig, Rat, Yeast
- **Published species reactivity**: Yeast, Pig, Rat, Insect, Non-human primate, Mollusc, Amphibian, Arthropod, Fish, Mouse, Human, Echinoderm, Not Applicable, Fruit fly

### Tested Applications
- **Gel Shift (GS)**: Assay dependent
- **Immunocytochemistry (ICC)**: 1:100
- **Immunofluorescence (IF)**: 1:50-1:200
- **Immunohistochemistry (Paraffin) (IHC (P))**: 1:200
- **Immunoprecipitation (IP)**: 2 µl
- **Western Blot (WB)**: 1:1000 - 1:5000

### Published Applications
- **Western Blot (WB)**: See 50 publications below
- **Miscellaneous PubMed (MISC)**: See 2 publications below
- **Immunohistochemistry (IHC)**: See 1 publications below
- **Immunocytochemistry (ICC)**: See 2 publications below
- **Neutralization (Neo)**: See 1 publications below
- **Immunoprecipitation (IP)**: See 2 publications below
- **Flow Cytometry (Flow)**: See 1 publications below
- **ELISA (ELISA)**: See 2 publications below

**Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.**

### Product specific information
MA3-006 detects several members of the heat shock protein 70 kDa (HSP70) gene family including HSP70, HSC70, p75, and following heat shock, HSP72 from yeast, Drosophila, fish, porcine, plant, mouse, avian, arthropod (red claw crayfish and black tiger prawn), amphibian, non-human primate, and human samples.

MA3-006 has been successfully used in Western blot, immunocytochemistry, immunofluorescence, gel shift, and immunoprecipitation procedures. By Western blot, this antibody detects proteins from ~70 kDa to ~78 kDa representing different members of the HSP70 family. 2-dimensional gel electrophoresis is required to resolve the heat induced form of these proteins from their constitutively expressed counterparts. Immunocytochemical staining of HSP70 in heat shocked HeLa cells with MA3-006 results in cytoplasmic staining.

The MA3-006 antigen is recombinant human HSP70 over-expressed in E. coli. Epitope mapping with a panel of HSP70 deletion mutants suggests that the epitope recognized is located between amino acids 504-617 of human HSP70, a region that has been shown to be involved in stress-induced nucleolar localization.


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Background/Target Information

Heat shock proteins (HSP) are expressed in response to various biological stresses, including high temperatures. There are several major families of HSPs including HSP70, HSP90 and HSP100. The HSP70 family is a set of highly conserved proteins that are induced by a variety of biological stresses, including heat stress, in every organism in which the proteins have been examined. The human HSP70 family members include: HSP70, a protein which is strongly inducible in all organisms but which is also constitutively expressed in primate cells; HSP72, a 72 kDa protein that is induced exclusively under stress conditions; HSC70, or cognate protein, is a 72 kDa, constitutively expressed, protein which is involved in the uncoating of clathrin coated vesicles; GRP78, or BiP, is a glucose regulated 78 kDa protein localized in the endoplasmic reticulum; and p75, or HSP75, a 75 kDa protein that is found within the mitochondria.

HSP70 Antibody (MA3-006) in IF
Immunofluorescent analysis of Heat Shock Protein 70 using Heat Shock Protein 70 Monoclonal antibody (3A3) (Product # MA3-006) shows staining in HeLa cells. Heat Shock Protein 70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing Heat Shock Protein 70 (Product # MA3-006) at a dilution of 1:100-1:200 over night at 4 °C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35552 for GAR, Product # 35503 for GAM). Images were taken at 60X magnification.

HSP70 Antibody (MA3-006) in IHC
Immunohistochemistry was performed on normal deparaffinized human Tonsil tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, 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 1:200 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 (Product # MA3-006) 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.

HSP70 Antibody (MA3-006) in WB
Western blot analysis of Heat Shock Protein 70 (Hsp70) was performed by loading 50ug of the indicated whole cell lysates and 15ul of PageRuler Prestained Protein Ladder (Product # 26616) onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a Hsp70 monoclonal antibody (Product # MA3-006) at a dilution of 1:1000 overnight at 4°C on a rocking platform, washed in TBS-0.1% Tween 20, and probed with a goat anti-mouse IgG-HRP secondary antibody (Product # 31430) at a dilution of 1:20,000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Pico (Product # 34080).

HSP70 Antibody (MA3-006) in IP
Immunoprecipitation of Heat Shock Protein 70 (Hsp70) was performed on HeLa cells. Antigen:antibody complexes were formed by incubating 500ug whole cell lysate with 2ug of Hsp70 monoclonal antibody (Product # MA3-006) overnight on a rocking platform at 4°C. The immune complexes were captured on 50ul Protein A/G Plus Agarose (Product # 20421), washed extensively, and eluted with Lane Marker Reducing Sample Buffer (Product # 39000). Samples were then resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a Hsp70 monoclonal antibody (Product # MA3-006) at a dilution of 1:1000 overnight rotating at 4°C, washed in TBS, and probed with goat anti-mouse IgG-HRP secondary antibody (Product # 31430) at a dilution of 1:20,000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura (Product # 34075).
HSP70 Antibody (MA3-006) in IF

Immunofluorescent analysis of Heat Shock Protein 70 (Hsp70) (green) in HeLa and NIH3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA (Product # 37525) for 15 minutes at room temperature. Cells were probed with a Hsp70 monoclonal antibody (Product # MA3-006), at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat-anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ArrayScan at 20X magnification.

HSP70 Antibody (MA3-006) in IHC

Immunohistochemistry was performed on cancer biopsies of deparaffinized human Breast carcinoma tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, 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 1:50 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 (Product # MA3-006) 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.

HSP70 Antibody (MA3-006) in WB

Western blot analysis of Hsp70 was performed by loading 20ul of gill tissue lysates from the salt marsh mussel, Guekensia demissa, isolated from various coves in Rhode Island (indicated above the lanes) and processed by homogenization in 32mM Tris-HCl, 1mM EDTA, and 2% SDS homogenization buffer, per well onto an SDS-PAGE gel. Proteins extracted from the supernatants were transferred to nitrocellulose membrane and blocked overnight with 5% milk in TBST. The membrane was probed with an Hsp70 monoclonal antibody (Product # MA3-006) at a dilution of 1:1000, washed in TBST, and probed with an HRP-conjugated goat anti-mouse IgG secondary antibody at a dilution of 1:3000. Detection was performed using SuperSignal West Pico (Product # 34080). Data courtesy of the Innovators Program.

HSP70 Antibody (MA3-006) in IF

Immunofluorescent analysis of Heat Shock Protein 70 using Heat Shock Protein 70 Monoclonal antibody (3A3) (Product # MA3-006) shows staining in NIH-3T3 cells. Heat Shock Protein 70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with an antibody recognizing Heat Shock Protein 70 (Product # MA3-006) at a dilution of 1:100-1:200 over night at 4 °C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35552 for GAR, Product # 35503 for GAM). Images were taken at 60X magnification.
HSP70 Antibody (MA3-006) in IF

Immunofluorescent analysis of Heat Shock Protein 70 using Heat Shock Protein 70 Monoclonal antibody (3A3) (Product # MA3-006) shows staining in MCF-7 cells. Heat Shock Protein 70 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing Heat Shock Protein 70 (Product # MA3-006) at a dilution of 1:100-1:200 over night at 4 °C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody (Product # 35552 for GAR, Product # 35503 for GAM). Images were taken at 60X magnification.

HSP70 Antibody (MA3-006) in IHC

Immunohistochemistry was performed on normal deparaffinized human Testis tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, 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 1:200 with a mouse monoclonal antibody recognizing Heat Shock Protein 70 (Product # MA3-006) 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.
### 50 Western Blot References

<table>
<thead>
<tr>
<th>Species / Dilution</th>
<th>Summary</th>
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<tbody>
<tr>
<td>Not Applicable / 1:2500</td>
<td>MA3-006 was used in western blot to study salinity and thermal stress in wild and farmed Pacific oysters Crassostrea gigas.</td>
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<tr>
<td></td>
<td>Comparative biochemistry and physiology. Part A, Molecular and integrative physiology (Nov 2016; 201: 22) <strong>&quot;Responses to thermal and salinity stress in wild and farmed Pacific oysters Crassostrea gigas.&quot;</strong></td>
</tr>
<tr>
<td></td>
<td>Author(s): Yang CY, Sierp MT, Abbott CA, Li Y, Qin JG \nPubMed Article URL: <a href="http://dx.doi.org/10.1016/j.cbpa.2016.06.024">http://dx.doi.org/10.1016/j.cbpa.2016.06.024</a></td>
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<td><strong>Not Applicable / 1:5000</strong></td>
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<td></td>
<td>MA3-006 was used in western blot to determine the effects of dendritic cell migration in vitro by loss of gadkin</td>
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<td>Author(s): Schachter H, Weimerhaus M, Stache V, Pleva N, Legler DF, Höpken UE, Maritzen T \nPubMed Article URL: <a href="http://dx.doi.org/10.1371/journal.pone.0143883">http://dx.doi.org/10.1371/journal.pone.0143883</a></td>
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<td></td>
<td><strong>Mouse / Not Cited</strong></td>
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<td>MA3-006 was used in western blot to study the temporal regulation of MyoD in skeletal muscle differentiation</td>
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<td>Developmental dynamics : an official publication of the American Association of Anatomists (Jan 2015; 244: 43) <strong>&quot;Contrasting roles for MyoD in organizing myogenic promoter structures during embryonic skeletal muscle development.&quot;</strong></td>
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<td>Author(s): Cho OH, Mallappa C, Hernández-Hernández JM, Rivera-Pérez JA, Imbalzano AN \nPubMed Article URL: <a href="http://dx.doi.org/10.1002/dvdy.24217">http://dx.doi.org/10.1002/dvdy.24217</a></td>
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<td><strong>Arthropod / 1:5000</strong></td>
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<td>MA3-006 was used in western blot to study the ability of a prickly pear cactus extract to increase Hsp70 levels and protect common carp against ammonia toxicity</td>
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<td>PLoS one (Sep 2013; 8: null) <strong>&quot;Non-lethal heat shock increased Hsp70 and immune protein transcripts but not Vibrio tolerance in the white-leg shrimp.&quot;</strong></td>
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<td>Author(s): Loc NH, Macrae TH, Musa N, Bin Abdullah MD, Abdul Wahid ME, Sung YY \nPubMed Article URL: <a href="http://dx.doi.org/10.1371/journal.pone.0073199">http://dx.doi.org/10.1371/journal.pone.0073199</a></td>
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<td>Author(s): Sung YY, Roberts RJ, Bossier P \nPubMed Article URL: <a href="http://dx.doi.org/10.1111/j.1365-2761.2012.01397.x">http://dx.doi.org/10.1111/j.1365-2761.2012.01397.x</a></td>
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<td><strong>Human / Not Cited</strong></td>
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<td>MA3-006 was used in western blot to investigate the important role of USP6 in regulation of cell migration and division</td>
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<td>Biology of the cell (Jan 2012; 104: 22) <strong>&quot;The oncogenic TBC domain protein USP6/TRE17 regulates cell migration and cytokinesis.&quot;</strong></td>
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<td></td>
<td>MA3-006 was used in western blot to detect immune system priming by heat shock protein 70</td>
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<tr>
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<td>Fish and shellfish immunology (Jul 2011; 31: 134) <strong>&quot;Priming the prophenoloxidase system of Artemia franciscana by heat shock proteins protects against Vibrio campbelli challenge.&quot;</strong></td>
</tr>
</tbody>
</table>
MA3-006 was used in western blot to study the effect of domestication on the stress response in juvenile Eurasian perch Perca fluviatilis

**Fish / 1:1000**

Comparative biochemistry and physiology. Part A, Molecular and integrative physiology (May 2011; 159: 92)

"Does domestication process affect stress response in juvenile Eurasian perch Perca fluviatilis?"


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Human / Not Cited

"NF-κB is not directly responsible for photoresistance induced by fractionated light delivery in HT-29 colon adenocarcinoma cells."

Author(s): Kulkova L, Mikeš J, Hyžalová M, Palumbo G, Fedoroko P

PubMed Article URL: http://dx.doi.org/10.1111/j.1751-1097.2010.00788.x

MA3-006 was used in western blot to investigate the effect of B-chromosome on heat shock protein expression in Eupreponemis plorans

**Insect / Not Cited**

Cytogenetic and genome research (Nov 2010; 132: 94)

"Level of heat shock proteins decreases in individuals carrying B-chromosomes in the grasshopper Eupreponemis plorans."

Author(s): Teruel M, Sørensen JG, Loeschecke V, Cabrero J, Perfectti F, Camacho JP

PubMed Article URL: http://dx.doi.org/10.1155/2010/319621

MA3-006 was used in western blot to investigate the effect of different Hsp70 on protection against Vibrio campbellii

**Arthropod / 1:5000**

Fish and shellfish immunology (Nov 2010; 29: 733)

"Efficacy of heterologous and homologous heat shock protein 70s as protective agents to Artemia franciscana challenged with Vibrio campbellii."

Author(s): Baruh K, Ranjan J, Sorgeoloos P, Bossier P

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MA3-006 was used in western blot to study the cellular changes in the black tiger shrimp Penaeus monodon in response to

**Fish / Not Cited**


"Do historical sediments of pulp and paper industry contribute to the exposure of fish caged in receiving waters?"

Author(s): Oikari A, Lahti M, Meriläinen P, Afanasiev S, Krasnov A

PubMed Article URL: http://dx.doi.org/10.1016/j.etap.2009.05.006

MA3-006 was used in western blot to study the effect of p53 expression on the efficacy of photodynamic therapy and colon cancer recurrence

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PubMed Article URL: http://dx.doi.org/10.1016/j.cbpa.2011.01.021

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"Oxidative stress, protein carbonylation and heat shock proteins in the black tiger shrimp, Penaeus monodon, following exposure to endosulfan and deltamethrin."

Author(s): Dorts J, Silvestre F, Tu HT, Tyberghein AE, Phuong NT, Kestemont P

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**Mouse / Not Cited**

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"Surface expression of a C-terminal alpha-helix region in heat shock protein 72 on murine LL/2 lung carcinoma can be recognized by innate immune sentinel."

Author(s): Tani F, Ōhno M, Furukawa Y, Sakamoto M, Masuda S, Kitabatake N

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MA3-006 was used in western blot to investigate the role of late embryogenesis abundant proteins in the drought response of Collembola

Journal of insect physiology (Mar 2009; 55: 210)

"Bioinformatics and protein expression analyses implicate LEA proteins in the drought response of Collembola."

Author(s): Bahndorf S, Tunnaciffe A, Wise MJ, McGee B, Holmstrup M, Loeschcke V
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"Sex-, gametogenesis, and tidal height-related differences in levels of HSP70 and metallothioneins in the Pacific oyster Crassostrea gigas."

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MA3-006 was used in western blot to investigate the influence of treated pulp mill and municipal effluents on papillomatisosis and heat shock protein 70 expression in Rutilus rutilus

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"The effects of treated effluents on the intensity of papillomatisosis and HSP70 expression in roach."

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Antonie van Leeuwenhoek (Dec 2007; 93: 205)

"Saccharomyces cerevisiae strains from traditional fermentations of Brazilian cachaça: trehalose metabolism, heat and ethanol resistance."

Author(s): Vianna CR, Silva CL, Neves MJ, Rosa CA
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MA3-006 was used in western blot to study how spawning affects thermotolerance in the Pacific oyster in summer with extremely high temperature

American journal of physiology. Regulatory, integrative and comparative physiology (Dec 2007; 293: R2353)

"Synergetic impacts of heat shock and spawning on the physiology and immune health of Crassostrea gigas: an explanation for summer mortality in Pacific oysters."

Author(s): Li Y, Qin JG, Abbott CA, Li X, Benkendorff K
PubMed Article URL: http://dx.doi.org/10.1152/ajpregu.00463.2007

MA3-006 was used in western blot to investigate the protective effect of sublethal heat shock on Crassostrea virginica with Perkinsus marinus infection against lethal heat stress

Diseases of aquatic organisms (Jul 2007; 76: 251)

"Heat shock protein (hsp70) expression and thermal tolerance in sublethally heat-shocked eastern oysters Crassostrea virginica infected with the parasite Perkinsus marinus."

Author(s): Encomio VG, Chu FL
PubMed Article URL: http://dx.doi.org/10.3354/dao076251

MA3-006 was used in western blot to study the influence of heat shock protein 70 on Chinese crab acclimation to cadmium

Bulletin of environmental contamination and toxicology (Jun 2007; 78: 432)

"Is HSP70 involved in acclimation to cadmium in the Chinese crab, Eriocheir sinensis?"

Author(s): Silvestre F, Trausch G, Devos P
PubMed Article URL: http://dx.doi.org/10.1007/s00128-007-9218-3
<table>
<thead>
<tr>
<th>Human / 1:3000</th>
<th>MA3-006 was used in western blot to study the effect of acclimation salinity on the stress protein response in two sibling species of Marenzelleria</th>
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<td>MA3-006 was used in western blot to study the role of in situ ATP synthesis in normal flagellar motility.</td>
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<td>MA3-006 was used in western blot to study the incorporation of a novel HSP40 into the radial spoke assembly during flagella assembly</td>
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<td>Fish / 1:3000</td>
<td>MA3-006 was used in western blot to investigate the effects of UV radiation on fish larvae</td>
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<table>
<thead>
<tr>
<th>Species / Dilution</th>
<th>Summary</th>
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<tr>
<td>Fish and shellfish immunology (Aug 2015; 45: 321)</td>
<td>MA3-006 was used in immunohistochemistry and western blot to study HSP70 in Apostichopus japonicus</td>
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</table>
| Echinoderm / 1:200 | "Histological, ultrastructural and heat shock protein 70 (HSP70) responses to heat stress in the sea cucumber Apostichopus japonicus."

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<thead>
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<th>Species / Dilution</th>
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<tr>
<td>Mouse / Not Cited</td>
<td>MA3-006 was used in immunocytochemistry and western blot to study heat shock protein expression and thermal tolerance in juvenile and adult King George whiting</td>
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<thead>
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<th>Summary</th>
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<tbody>
<tr>
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</table>
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<tbody>
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</table>
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<tr>
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<tr>
<td>Non-human primate / Not Cited</td>
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