**Product datasheet**

**Anti-NFAT5 antibody - ChIP Grade ab3446**

| Overview |
|-----------------|-----------------|
| **Product name** | Anti-NFAT5 antibody - ChIP Grade |
| **Description** | Rabbit polyclonal to NFAT5 - ChIP Grade |
| **Host species** | Rabbit |
| **Specificity** | Detects Nuclear Factor of Activated T-cells 5 (NFAT 5). |
| **Tested applications** | Suitable for: ICC, WB, ChIP, ICC/IF, IP, IHC-P, Immunomicroscopy |
| **Species reactivity** | Reacts with: Mouse, Rat, Human, Pig |
| **Immunogen** | Synthetic peptide corresponding to Human NFAT5 aa 1439-1455 (C terminal). Sequence: DLLVSLQNGNLTGSF |
| | (Peptide available as ab4978) |

<table>
<thead>
<tr>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Form</strong></td>
</tr>
<tr>
<td><strong>Storage instructions</strong></td>
</tr>
<tr>
<td><strong>Storage buffer</strong></td>
</tr>
<tr>
<td><strong>Purity</strong></td>
</tr>
<tr>
<td><strong>Clonality</strong></td>
</tr>
<tr>
<td><strong>Isotype</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our <a href="#">Abpromise guarantee</a> covers the use of ab3446 in the following tested applications.</td>
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<td>The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.</td>
</tr>
</tbody>
</table>
**Function**
Plays a role in the inducible expression of genes. Regulates hypertonicity-induced cellular accumulation of osmolytes.

**Tissue specificity**
Highest levels in skeletal muscle, brain, heart and peripheral blood leukocytes. Also expressed in placenta, lung, liver, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine and colon.

**Sequence similarities**
Contains 1 RHD (Rel-like) domain.

**Cellular localization**
Nucleus.

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**Images**

Immunoprecipitation of NFAT5 was performed on U2OS cells. The antigen:antibody complex was formed by incubating 500µg whole cell lysate with 3µg of ab3446 overnight on a rocking platform at 4°C. The immune-complex was captured on 50µl Protein A/G Plus Agarose. Captured immune-complexes were washed and proteins eluted with 5X Reducing Sample Loading Dye. Samples were resolved on a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to PVDF membrane and blocked with 5% Milk/TBS-0.1%Tween for at least 1 hour. Membranes were washed in TBS-0.1%Tween 20 and probed with a goat anti-rabbit-HRP secondary antibody at a dilution of 1:20,000 for at least one hour. Membranes were washed and chemiluminescent detection performed.
Immunocytochemistry/Immunofluorescence analysis of NFAT5 in HeLa cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature. Cells were then blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with ab3446 at a dilution of 1:100 for at least 1 hour at room temperature. Cells were washed with PBS and incubated with DyLight 488 goat-anti-rabbit secondary antibody at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye. Images were taken at 20X magnification.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) was performed on normal biopsies of deparaffinized human skeletal muscle 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 incubated with ab3446 at a dilution of 1:20 (left) or without primary antibody (negative control - right) 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.

Western blot analysis of NFAT5 was performed by loading 25ug of various whole cell lysates onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% Milk/TBST for at least 1 hour. Membranes were incubated with ab3446 at a dilution of 1:1000 overnight at 4°C on a rocking platform. Membranes were washed in TBS-0.1% Tween 20 and probed with a goat anti-rabbit-HRP secondary antibody at a dilution of 1:20,000 for at least one hour. Membranes were washed and chemiluminescent detection performed.
Immunocytochemistry/Immunofluorescence analysis of NFAT5 in HeLa Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control - right) or with ab3446 at a dilution of 1:20 overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. NFAT5 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) was performed on normal biopsies of deparaffinized human brain 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 incubated with ab3446 at a dilution of 1:20 (left) or without primary antibody (negative control - right) 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.

Western blot of Human NFAT5 from transfected BHK cell lysate with ab3446.
Immunocytochemistry/Immunofluorescence analysis of NFAT5 in NIH-3T3 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control - right) or with ab3446 at a dilution of 1:20 overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. NFAT5 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.

Immunohistochemical analysis of rat spinal tissue, staining NFAT5 with ab3446. Sections were incubated with primary antibody (1/100) overnight at 4°C before incubating with a biotinylated secondary antibody. Staining was detected using DAB.

Immunocytochemistry/Immunofluorescence analysis of NFAT5 in MCF-7 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control - right) or with ab3446 at a dilution of 1:200 overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. NFAT5 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.

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