Technical Data Sheet

Purified Mouse Anti-NSF

### Material Information
- **Material Number:** 612272
- **Size:** 50 µg
- **Concentration:** 250 µg/ml
- **Clone:** 7/NSF
- **Immunogen:** Human NSF aa. 488-601
- **Isotype:** Mouse IgG2b
- **QC Testing:** Human
- **Reactivity:** Tested in Development: Mouse, Rat, Dog, Chicken
- **Target MW:** 82 kDa
- **Storage Buffer:** Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

### Description
Eukaryotic protein trafficking involves the packaging of target molecules into membraneous vesicles that bud from a donor compartment, travel to a specific destination, fuse, and release their components into an acceptor compartment. Components of both the vesicle and the plasma membrane interact to form a fusion complex, which mediates specific docking and fusion of vesicles. This complex contains NSF (N-ethyl-maleimide-sensitive factor), SNAPs (soluble NSF attachment proteins), and receptor proteins (SNAREs). SNAP-25 (synaptosome-associated protein of 25kDa) and syntaxin are SNAREs that are associated with the target plasma membrane (t-SNAREs), while synaptobrevin and synaptotagmin are vesicle-associated SNARE proteins (v-SNAREs). SNAREs form a 7S complex that has a high affinity binding site for α-SNAP and NSF. Upon binding of α-SNAP and NSF, a 20S complex is formed that is rapidly disassembled due to NSF’s ATPase activity. This facilitates vesicle fusion to the target membrane followed by fusion and release of vesicle components.

### Western Blot Analysis
**Western blot analysis of NSF on a HeLa lysate (left).** Lane 1: 1:5000, lane 2: 1:10000, lane 3: 1:20000 dilution of the anti-NSF antibody.

### Immunofluorescent Staining
**Immunofluorescent staining of SK-N-SH cells (right).** Cells were seeded in a 384 well collagen coated Microplates (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure; Bioimaging protocol link) and the anti-NSF antibody. The second step reagent was Alexa Fluor® 488 goat anti mouse Ig (Invitrogen)(pseudo colored green). Cell nuclei were counter stained with Hoechst 33342 (pseudo colored blue). The image was taken on a BD Pathway™ 855 or 43S Bioimager System using a 20x objective and merged using the BD AttoVison ™ software. This antibody also stained SH-SYSY, C6, U87 and U373 cells using both the Triton X100 and methanol fix/perm protocols (see Recommended Assay Procedure; Bioimaging protocol link).

### Preparation and Storage
Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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612272 Rev. 2
**Application Notes**

**Application**

<table>
<thead>
<tr>
<th>Method</th>
<th>Tested During Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western blot</td>
<td>Routinely Tested</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>Tested During Development</td>
</tr>
<tr>
<td>Bioassay</td>
<td>Tested During Development</td>
</tr>
</tbody>
</table>

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>611449</td>
<td>HeLa Cell Lysate</td>
<td>500 µg</td>
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<tr>
<td>554001</td>
<td>FITC Goat Anti-Mouse Ig</td>
<td>0.5 mg</td>
<td>Polyclonal</td>
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<tr>
<td>554002</td>
<td>HRP Goat Anti-Mouse Ig</td>
<td>1.0 ml</td>
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**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
5. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. Triton is a trademark of the Dow Chemical Company.

**References**

