Accurate chromosome segregation requires that all pairs of sister chromatids become appropriately attached to mitotic spindles before the onset of anaphase. Cell cycle checkpoints monitor kinetochore-microtubule interactions, so that cell cycle progression can be delayed until proper chromosome attachments are formed. In yeast, *Bub1-3* genes are required for proper mitotic delay in response to unattached kinetochores. In mammals, the homologues to yeast *Bub1* and *Bub3* form a complex that binds kinetochores and has protein kinase activity. *Bub3* contains four WD repeats, three in the N-terminus and one in the C-terminus, and a central *Bub1*-binding domain. During prophase and prometaphase, *Bub3* localizes to the kinetochore before attachment to microtubules. In addition, taxol-induced formation of lagging chromosomes due to a delay of cell cycle progression increases the level of *Bub3* co-localized with kinetochores, while correctly aligned chromosomes found in metaphase do not exhibit this co-localization. Thus, *Bub3*, in association with *Bub1*, may be important for sensing kinetochore attachment to microtubules during the prometaphase to metaphase transition.

**Preparation and Storage**

Store undiluted at -20°C. The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Application Notes

Application

<table>
<thead>
<tr>
<th>Application</th>
<th>Routinely Tested</th>
<th>Tested During Development</th>
</tr>
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<tbody>
<tr>
<td>Western blot</td>
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<tr>
<td>Bioimaging</td>
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Recommended Assay Procedure:

**Bioimaging**

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.

2. Remove the culture medium from the wells, and fix the cells by adding 100 μl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).

3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 μl of ~20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
   OR
   b. Add 100 μl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.

4. Remove the permeabilization buffer, and wash the wells twice with 100 μl of 1× PBS.

5. Remove the PBS, and block the cells by adding 100 μl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.

6. Remove the blocking buffer and add 50 μl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.

7. Remove the primary antibody, and wash the wells three times with 100 μl of 1× PBS.

8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 μl to each well, and incubate in the dark for 1 hour at RT.

9. Remove the second step reagent, and wash the wells three times with 100 μl of 1× PBS.

10. Remove the PBS, and counter-stain the nuclei by adding 200 μl per well of 2 μg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.

11. View and analyze the cells on an appropriate imaging instrument.

**Bioimaging:** For more detailed information please refer to [http://www.bdbiosciences.com/support/resources/protocols/confirm_reagents.jsp](http://www.bdbiosciences.com/support/resources/protocols/confirm_reagents.jsp)

**Western blot:** For more detailed information please refer to [http://www.bdbiosciences.com/support/resources/protocols/monoclonal_anti.jsp](http://www.bdbiosciences.com/support/resources/protocols/monoclonal_anti.jsp)

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554001</td>
<td>FITC Goat Anti-Mouse Ig</td>
<td>0.5 mg</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>554002</td>
<td>HRP Goat Anti-Mouse Ig</td>
<td>1.0 ml</td>
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<tr>
<td>353219</td>
<td>BD Falcon™ 96-well Imaging Plate</td>
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<td>(none)</td>
</tr>
<tr>
<td>554655</td>
<td>Fixation Buffer</td>
<td>100 ml</td>
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<tr>
<td>558050</td>
<td>Perm Buffer III</td>
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<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
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<td>(none)</td>
</tr>
</tbody>
</table>

**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Triton is a trademark of the Dow Chemical Company.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.

**References**
