Technical Data Sheet

Purified Mouse Anti-Laminin B2

Product Information

- **Material Number:** 610722
- **Size:** 50 µg
- **Concentration:** 250 µg/ml
- **Clone:** 22/Laminin
- **Immunogen:** Human Laminin B2 aa. 506-691
- **Isotype:** Mouse IgG1
- **Reactivity:** QC Testing: Human
  Tested in Development: Rat, Rabbit
- **Target MW:** 220 kDa
- **Storage Buffer:** Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The basal lamina contains Collagen Type IV, proteoglycans, and glycoproteins. Laminin is a high molecular weight (~850 kDa) oligomer, consisting of three different chains (A, B1, and B2) joined by disulfide bonds. The overall structure predicts two helical domains (I&II) at the COOH-terminal, cysteine-rich repeats (III&V), and two globular domains (IV&VI). Domains IV and V1 are the binding sites for collagen and heparan sulfate, respectively. Several isoforms have been identified for the genes of each chain. Laminin B2 is 1576 amino acids plus a 33 amino acid signal peptide, 14 glycosylation sites, and 12 cysteine repeat domains. The expression of the Laminin subunits is detected rapidly during development and tissue regeneration. Attachment of cells to the basal lamina affects mitogenesis, motility, and differentiation.

![Western blot analysis of Laminin B2 on a HepG2 lysate (Cat. No. 611555). Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the Laminin B2 antibody.](image1)

![Immunofluorescent staining of A549 (ATCC CCL-185) cells. Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-Laminin B2 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96)cells and can be used with either perm protocol (see Recommended Assay Procedure).](image2)

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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Application Notes

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<th>Application</th>
<th>Tested During Development</th>
<th>Routinely Tested</th>
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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/certified_reagents.jsp

**Western blot:** For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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</thead>
<tbody>
<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>
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<tr>
<td>611555</td>
<td>HepG2 Cell Lysate</td>
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<tr>
<td>353219</td>
<td>BD Falcon™ 96-well Imaging Plate</td>
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<td>(none)</td>
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<tr>
<td>554655</td>
<td>Fixation Buffer</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>
<td>500 ml</td>
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**Product Notices**
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. 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.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

**References**
Engvall E. Laminin variants: why, where and when?. Kidney Int. 1993; 43(1):2-6. (Biology)