Purified Mouse Anti-Phospholipase Cγ1

**Product Information**

- **Material Number:** 610027
- **Size:** 50 µg
- **Concentration:** 250 µg/ml
- **Clone:** 10/PLCgamma
- **Immunogen:** Cow PLCγ1 N-terminal region Peptide
- **Isotype:** Mouse IgG1
- **Reactivity:** Tested in Development: Mouse, Rat, Dog, Chicken
- **Target MW:** 148 kDa
- **Storage Buffer:** Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

**Description**

The Phospholipase C (PLC) isozymes hydrolyze phosphatidyl inositol biphosphate to inositol triphosphate and diacylglycerol. The former causes release of calcium from the endoplasmic reticulum, while the latter is an activator of Protein Kinase C. Within the PLC family, PLCγ is the only member that contains SH2 and SH3 domains. These domains enable it to interact with receptor tyrosine kinases and become enzymatically activated via phosphorylation. It exists as two isoforms: 1) PLCγ1, which is ubiquitously expressed, and 2) PLCγ2, found primarily in the lymphoid system. PLCγ is essential for growth factor-induced cell motility and mitogenesis. PLCγ1 null mice exhibit retarded embryonic growth and lethality in midgestation. Overexpression of PLCγ is evident in several forms of cancer, and it has been identified as a key mediator of PDGF-dependent cellular transformation. Thus regulation of PLCγ activity by growth factors is involved in cell growth and transformation.

The 10/PLCgamma monoclonal antibody recognizes PLCγ1, regardless of phosphorylation status. It does not cross-react with PLCγ2.

**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**

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<thead>
<tr>
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<td>Western blot</td>
<td>Routinely Tested</td>
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<tr>
<td>Bioimaging</td>
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<td>Immunohistochemistry</td>
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**Recommended Assay Procedure:**

**Western blot**: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

**Recommended Protocol for Bioimaging:**

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.
2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.
3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 µl of 1× PBS.
5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c):
   a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
   c. Add 100 µl of 1× Perm/Wash buffer (Cat. No. 554723) to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.
6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 µl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.).
7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.
8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.
9. Add 50 µl of diluted antibody per well and incubate for 120 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.
10. Remove the antibody, and wash the wells three times with 100 µl of wash buffer. An optional detergent wash (100 µl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.
11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.
12. After the final wash, counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg. Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
13. View and analyze the cells on an appropriate imaging instrument. Recommended filters for the BD Pathway™ instruments are:

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<th>Instrument</th>
<th>Excitation</th>
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<tr>
<td>BD Pathway 855</td>
<td>488/10</td>
<td>515 LP</td>
<td>Fura/FITC</td>
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<tr>
<td>BD Pathway 435</td>
<td>482/35</td>
<td>536/40</td>
<td>FF506</td>
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**Suggested Companion Products**

<table>
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<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>611447</td>
<td>A431 Cell Lysate</td>
<td>500 µg</td>
<td>(none)</td>
</tr>
<tr>
<td>554002</td>
<td>HRP Goat Anti-Mouse Ig</td>
<td>1.0 ml</td>
<td>(none)</td>
</tr>
<tr>
<td>554001</td>
<td>FITC Goat Anti-Mouse Ig</td>
<td>0.5 mg</td>
<td>Polyclonal</td>
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**Product Notices**

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

**References**


Obermeier A, Tinhofer I, Grunicke HH, Ullrich A. Transforming potentials of epidermal growth factor and nerve growth factor receptors inversely correlate with their phospholipase C gamma affinity and signal activation. *EMBO J.* 1996; 15(1):73-82. (Biology)