Protocol-at-a-Glance

# Living Colors® DsRed Monoclonal Antibody Protocol-at-a-Glance

**(PT3647-2)**

The Living Colors DsRed Monoclonal Antibody (Cat. Nos. 632392, 632393) is recommended for use in Western blotting analysis. The antibody recognizes denatured forms of wild-type DsRed and its variants, including DsRed1, DsRed2, and DsRed1-E5, the Fluorescent Timer (1–3). The antibody binds DsRed1 and DsRed2 even when these proteins are expressed as fusions to other proteins. Both N- and C-terminal fusions are recognized by the antibody.

For best results with this antibody, we suggest you use the Western blotting procedure given in this Protocol-at-a-Glance. It describes how to prepare mammalian cell lysate for SDS/polyacrylamide gel electrophoresis (SDS-PAGE) and how to analyze a polyacrylamide gel by Western blotting. In following this protocol, please refer to the Product Analysis Certificate included with your antibody for antibody-specific information such as recommended dilutions.

*PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.*

## A. Materials Required

- **1X PBS**
- **2X SDS sample buffer** (4)
- **SDS/polacrylamide gel**
- **Nitrocellulose or PVDF membrane** (for gel blotting)
- **Wash buffer** (0.1% Tween-20; 150 mM NaCl; 10 mM Tris-HCl, pH7.5)
- **Blocking buffer** (5% nonfat dry milk in wash buffer)

  It may be necessary to warm the solution slightly to fully dissolve nonfat dry milk. Store at 4°C. (Blocking buffer is stable for up to 3 days when properly stored. Do not add $NaN_3$ if you plan to use horseradish peroxidase (HRP)-conjugated secondary antibody; $NaN_3$ inactivates HRP.)
- **Secondary antibody**

  Use either an alkaline phosphatase (AP) or horseradish peroxidase (HRP) conjugated secondary antibody directed against your primary antibody, mouse IgG.
- **AP or HRP detection system** (colorimetric or chemiluminescent)

## B. Preparation of Mammalian Cell Lysate and Gel Electrophoresis

1. Collect 1–2 x $10^6$ log-phase cells in a 15-ml conical centrifuge tube.

   **Note:** For each cell culture being analyzed, you will need to load ~20 µg of total protein in a single lane of the SDS/polyacrylamide gel. A suspension containing 1 x $10^6$ cells typically yields ~200 µg of total protein.

   a. To collect adherent cells, treat one 100-mm culture plate (~80% confluent) with trypsin.

   b. Resuspend trypsinized cells in 5 ml of chilled 1X PBS.
2. Centrifuge the cell suspension at 2,000 rpm for 10 min at 4°C.
3. Remove the supernatant. Then gently resuspend the pellet in 5 ml of chilled PBS.
4. Centrifuge the cell suspension at 2,000 rpm for 10 min at 4°C.
5. Remove the supernatant. Then lyse cells using your method of choice. Following are three suggested methods for preparing **total cell lysate:**

   **Note**: To protect your fusion protein against proteolytic degradation, include protease inhibitors in the lysis buffer.

   (1) Lysis by sonication

   a. Resuspend cells in an appropriate volume of chilled PBS.

   b. Disrupt cells by sonication.

   c. Proceed to Step 7, or go to Step 6 to prepare a PNS (post-nuclear supernatant) fraction.

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(2) Lysis by rapid extrusion

a. Resuspend cells in an appropriate volume of chilled PBS.

b. Disrupt cells by repeatedly passing the suspension through a 21-gauge needle.

c. Proceed to Step 7, or go to Step 6 to prepare a PNS (post-nuclear supernatant) fraction.

(3) Lysis by emulsification

a. Resuspend cells in an appropriate volume of 2X SDS sample buffer.

b. Mix by pipetting up and down.

**Note:** If the lysate is too viscous, add a small volume of 5X SDS sample buffer.

c. Proceed to Step 7.a.

6. To prepare a **PNS (post-nuclear supernatant) fraction**:
   a. Centrifuge the total cell lysate (prepared by mechanically disrupting cells—e.g., by sonicating cells) at 500 x g for 10 min at 4°C.
   b. Collect the supernatant. (Discard the pellet, which contains cell nuclei.)
   c. Measure the protein concentration using standard techniques.
   d. Mix 20 µl (about 20 µg of protein) of the supernatant with an equal volume of 2X SDS sample buffer.
   e. Heat at 100°C for 5 min.
   f. Proceed to Step 8.
7. Add an appropriate volume of 2X SDS sample buffer.
   a. Heat the sample at 100°C for 5 min.
   b. Centrifuge the lysate at 10,000 rpm for 10 min at 4°C.
8. Load the desired volume of boiled lysate on a polyacrylamide gel and perform electrophoresis using standard procedures.

### C. Western Blotting

**Note:** Optimal dilutions and incubation times may vary with individual systems and must be determined empirically.

1. Transfer proteins from the gel to a nitrocellulose or PVDF membrane using standard techniques.
2. Add 20 ml blocking buffer and incubate membrane for 1 hr at room temperature with shaking. Alternatively, incubate at 4°C overnight.
3. Dilute the Living Colors DsRed Monoclonal Antibody in blocking buffer according to the specifications on the Product Analysis Certificate.
4. Incubate the membrane with the diluted antibody for 2 hr at room temperature with shaking.
5. Wash the membrane two times with wash buffer for 5 min each wash with shaking.
6. Dilute a secondary antibody conjugate in blocking buffer according to the manufacturer's specifications.
7. Incubate the membrane with the diluted antibody for 1 hr at room temperature with shaking.
8. Wash the membrane four times with wash buffer for 10 min each wash.
9. Proceed with an appropriate chemiluminescent or colorimetric detection method.

## References


1. Living Colors DsRed2 (July 2001) *CLONTECHniques* **XVI**(3):2–3.
2. Living Colors Fluorescent Timer (April 2001) *CLONTECHniques* **XVI**(2):14–15.
3. Matz, M. V., Fradkov, A. F., Labas, Y. A., Savitsky, A. P., Zaraisky, A. G., Markelov, M. L. & Lukyanov, S. A. (1999) Fluorescent proteins from nonbioluminescent *Anthozoa* species. *Nature Biotech.* **17**:969–973.
4. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratories, Cold Spring Harbor, NY).



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