

Product Number  
**926-68100**

Quantity: 25 blots

Storage: 4°C

Revised: April-2012

Updates available at:

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Doc #988-12974



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## Quick Western Kit – IRDye® 680RD

### Applications

The Quick Western Kit – IRDye 680RD provides a universal antibody detection reagent that can be combined with the primary antibody incubation step, eliminating the need for a secondary antibody. The overall time to complete a Western blot is reduced while providing the advantages of near infrared detection. The kit can be used to detect primary antibodies from a variety of hosts and has been shown to recognize primary antibodies to recombinant tagged proteins (e.g. 6X His, Myc, FLAG, etc.).

The kit also serves as a detection solution for post-immunoprecipitation samples by Western blot because it does not bind to denatured mouse monoclonal or rabbit monoclonal antibodies. The key benefit is the ability to use the same antibody for immunoprecipitation and post-immunoprecipitation detection by Western blot.

For additional protocols, please reference the Odyssey® or Aerius Imaging System Applications Manuals included with all instrument installations, or download from <http://biosupport.licor.com>.

### Kit Components

- IRDye 680RD Detection Reagent
- Odyssey Blocking Buffer
- Quick Reference Card

### Additional Reagents Required

- 1X PBS
- Primary Antibody
- 1X PBS-T (1X PBS containing 0.1% Tween® 20)
- 20% Tween 20
- 20% SDS (If using PVDF membrane)

### IRDye 680RD Detection Reagent Properties

**Fluorophore:** IRDye 680RD

**Excitation Wavelength:** 676 nm (in PBS)

**Emission Wavelength:** 694 nm (in PBS)

**Form of Detection Reagent:** IRDye 680RD Detection Reagent, lyophilized in water. Contains 10 mg BSA (IgG and protease free) per mg of Detection Reagent as a stabilizer and 0.01% sodium azide as a preservative.

**Warning:** *Sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.*

### Purity and Specificity

IRDye 680RD Detection Reagent is known to have high affinity for IgG from human, mouse, rabbit, guinea pig, goat, sheep, pig, cow, cat, dog, and donkey. The Detection Reagent is known to have lower affinity for mouse IgG<sub>1</sub>, mouse IgA, rat, horse, and hamster IgGs and will not detect primary antibodies from a chicken host. The Detection Reagent has been specifically tested and qualified for Western blot applications. If additional affinity is required, please use IRDye conjugated secondary antibodies for detection.

### Reconstitution and Storage

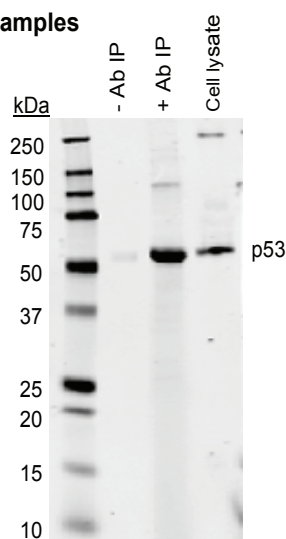
**Protect from light.** Store at 4°C. Reconstitute contents of vial with 0.25 mL 1X PBS. Mix gently by inverting, and allow to rehydrate for at least 60 minutes (protect from light) before use. Centrifuge product if solution is not completely transparent after standing at room temperature. This product is stable for up to 3 months at 4°C when stored in the reconstituted form.

## Western Detection Protocol – Quick Western Kit

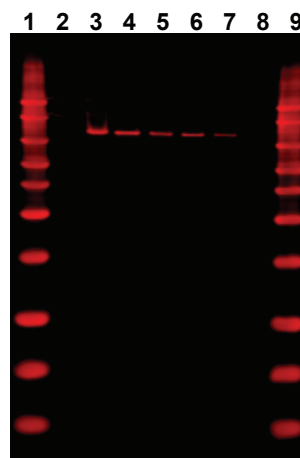
1. Pre-wet Membranes
  - **Nitrocellulose membranes:** pre-wet in 1X PBS.
  - **PVDF membranes:** briefly pre-wet in 100% methanol and rinse with ultrapure water before wetting in PBS.
2. Block the membrane:
  - Block the membrane with Odyssey<sup>®</sup> Blocking Buffer for 1 hour\* at room temperature  
*\*Shorter blocking times (e.g. 30 min) may provide equivalent results*
  - Use enough blocker to cover membrane (e.g. 10 mL for a 7 x 8 cm membrane)
3. Prepare Detection Solution:  
*Primary Antibody and IRDye<sup>®</sup> 680RD Detection Reagent in Odyssey Blocking Buffer*
  - Add primary antibody (dilute as recommended by vendor) to Odyssey Blocking Buffer
  - Add the IRDye 680RD Detection Reagent to the primary antibody + Odyssey Blocking Buffer solution
  - Add 1 µL of Detection Reagent to every 1 mL of Odyssey Blocking Buffer (1:1000 dilution)
  - Add detergent:
    - Nitrocellulose membranes: 0.2% Tween<sup>®</sup> 20\* to the Detection Solution
    - PVDF membranes: 0.2% Tween 20\* and 0.02% SDS\* to the Detection Solution

*\*Note: Optimization is required to determine final concentrations of detergents. Concentrations can range from 0.1 – 0.2% Tween 20 and 0.02 – 0.1% SDS.*
4. Decant and discard blocker.
5. Add Detection Solution (prepared in step 3)
6. Incubate using your standard primary antibody incubation time, at room temperature on a platform shaker; protect from light.
7. Wash Membrane
  - Decant antibody solution and rinse blot briefly in 15 mL of 1X PBS-T (1X PBS + 0.1% Tween 20).
  - Decant.
  - Add 15 mL of 1X PBS-T and incubate at room temperature on platform shaker protected from light for 5 minutes.
  - Decant.
  - Repeat wash step 2 more times.
8. Rinse membrane with 15 mL of 1X PBS.
9. Scan membrane (wet or dry) on an Odyssey or Aeries Imaging System using the 700 nm detection channel.
  - Instrument settings may need to be optimized for best image appearance. Adjust scan intensity settings, as needed.

### Examples



A431 cell lysates were immunoprecipitated overnight with a monoclonal antibody against p53. The resulting immunoprecipitates were separated by SDS-PAGE. Lane 1: Negative IP control; Lane 2: Test sample; Lane 3: A431 cell lysate positive control. Western blotting was performed using the same p53 monoclonal antibody and incubated with IRDye 680RD Immunoprecipitation Detection Reagent.



Two-fold dilutions of crude lysate containing an over-expressed 6X-His tagged DNA polymerase were loaded in Lanes 3-7. A His-tag molecular weight marker (Invitrogen) was loaded in Lanes 1 & 9. The nitro-cellulose membrane was blocked with Odyssey Blocking Buffer and probed with anti-His Tag Rabbit Polyclonal Antibody (GenScript; 1:500) and IRDye 680RD Detection Reagent (1:1000) for 1 hour. The image was collected on Odyssey CLx.