LI-COR®

Revert™ 700 Total Protein Stain Pack Insert

Kit Components

P/N	Description
926-11010	**Revert 700 Total Protein Stain Kit, 100 mL** Sufficient reagent for up to 20 mini blots. Revert 700 Total Protein Stain, 100 mL Revert 700 Wash Solution, 200 mL Revert Destaining Solution, 200 mL
926-11016	**Revert 700 Total Protein Stain Kit, 250 mL** Sufficient reagent for up to 50 mini blots. Revert 700 Total Protein Stain, 250 mL Revert 700 Wash Solution, 500 mL Revert Destaining Solution, 500 mL
926-11015	**Revert 700 Total Protein Stain and Wash Solution Kit** Sufficient reagent for up to 20 mini blots. Revert 700 Total Protein Stain, 100 mL Revert 700 Wash Solution, 200 mL
926-11014	**Revert 700 Sample Pack** Sufficient reagent for up to 4 mini blots. Revert 700 Total Protein Stain, 20 mL Revert 700 Wash Solution, 40 mL Revert Destaining Solution, 40 mL
926-11011	**Revert 700 Total Protein Stain, 100 mL** Sufficient reagent for up to 20 mini blots.
926-11021	**Revert 700 Total Protein Stain, 250 mL** Sufficient reagent for up to 20 mini blots.
926-11012	**Revert 700 Wash Solution, 200 mL** Sufficient reagent for up to 20 mini blots.
926-11013	**Revert Destaining Solution, 200 mL** Sufficient reagent for up to 20 mini blots.

Specifications

- Shelf Life: 6 months from date of receipt
- Wash Solution: 6.7% (v/v) glacial acetic acid, 30% (v/v) methanol, in water. Revert Destaining Solution: 0.1 M sodium hydroxide, 30% (v/v) methanol, in water.
- Membrane Types: Nitrocellulose or PVDF

Product Description

Revert 700 Total Protein Stain is a membrane stain that fluoresces at 700 nm and does not covalently modify sample proteins and therefore does not affect antibody binding or quantification. Revert 700 Total Protein Stain provides high signal intensity with a broad, linear quantitative range. Stained proteins produce signal that can be detected visibly and by imagers capable of detecting dyes that have excitation and emission maxima near 700 nm. With an imaging system capable of multiplex detection, you can image the total protein stained with Revert 700 Total Protein Stain in the 700 nm channel and image a target protein in the 800 nm channel on the same blot. You can also remove the total protein stain and process the blot to detect two different targets in the 700 and 800 nm channels.

With a total protein stain, you will be able to monitor protein transfer across the entire blot at all molecular weights. This will allow you to determine if there are any irregularities that indicate you should run the blot again to get more robust results.

Applications

In Western blot analysis, normalization is essential for accurate, reproducible comparison of protein levels. Validating housekeeping proteins that are suitable for Western blot normalization adds significant time and cost to experiments. The total protein normalization method using Revert 700 Total Protein Stain provides a quick and reliable method for normalizing target signals.

Prepare Western Blot

Method 1: Single-Color Western Blot (800 nm target only)

Use the steps below for detection of a target in the 800 nm channel.

Step 1. Stain with Revert™ 700 Total Protein Stain

1. Add methanol to the stain reagents as indicated on each bottle.
2. After transfer is complete, fully dry the membrane. Place the membrane on top of a piece of clean filter paper and allow it to dry:
    - 40 to 60 minutes at room temperature.
    - 10 minutes in an oven at 37 °C.
    - Overnight at room temperature as a stopping point.

3. Rehydrate the membrane after fully drying.
    - For nitrocellulose membranes, incubate the membrane in TBS or PBS (no detergent) for 5 minutes at room temperature with gentle shaking.
    - For PVDF membranes, first rehydrate using 100% methanol for 30 seconds. Then rinse in TBS or PBS (no detergent) for 5 minutes at room temperature with gentle shaking.
4. Rinse the membrane with ultrapure water.

    Note: *Before moving to the next step, ensure the membrane container provides a minimum clearance of 1/8th of an inch on all sides. Revert 700 Total Protein Stain will cause the membrane to swell. Without clearance, staining may be uneven.*
5. Stain membrane with Revert 700 Total Protein Stain. Incubate the membrane in 5 mL of Revert 700 Total Protein Stain solution for 5 minutes at room temperature with gentle shaking.

    Note: *Do not allow the membrane to dry from this point on.*
6. Decant total protein stain solution thoroughly. Using approximately 5 mL of Revert 700 Wash Solution (P/N 926-11012), rinse the membrane two times for 30 seconds at room temperature with gentle shaking.
7. Decant wash solution thoroughly, then briefly rinse the membrane with ultrapure water.

Step 2. Image Membrane

Note: *Do not allow the membrane to dry during imaging. If you are using an Odyssey® DLx or an Odyssey® M, it is best to place the silicone mat on top of the membrane. See the Operator's Manual for your imager for detailed instructions (licor.com/support).*

1. Immediately image the membrane in the 700 nm channel using an Odyssey® Imaging System. If saturation occurs, reduce the scan intensity or acquisition time, or use AutoScan if your instrument includes this.
2. Proceed immediately to blocking and follow your normal Western blot protocol using IRDye® 800CW Secondary Antibody to detect your target in the 800 nm channel.
3. Image the membrane in the 800 nm channel with an Odyssey® Imaging System. If saturation occurs, reduce the scan intensity or acquisition time, or use AutoScan if your instrument includes this.

    Note: *Visible color from stain will wash off during processing and residual total protein signal may be detected in the 700 nm channel.*

Method 2: Two-Color Western Blot (700 and 800 nm targets)

Use the steps below for detection of targets in the 700 nm and 800 nm channels. Follow the single-color Western blot steps up until imaging the membrane with Revert™ 700 Total Protein Stain in the 700 nm channel (step 2.1). After imaging the membrane, proceed to the destaining step.

Step 1. Destaining

1. Briefly rinse membrane with ultrapure water.

   Note: *This step is important for the destaining step to work properly.*

2. Incubate the membrane in 5 mL of Revert Destaining Solution (P/N 926-11013) for 5 to 10 minutes, with gentle shaking. Destaining is complete when stain is no longer visible by eye.

   Warning: *Do not destain for longer than 10 min.*

Step 2. Process Western Blot

1. Decant destaining solution thoroughly, then briefly rinse the membrane with ultrapure water. Proceed immediately to blocking and immunodetection.

2. Follow your normal Western blot protocol using IRDye® 800CW Secondary Antibody to detect your target in the 800 nm channel and IRDye 680RD Secondary Antibody to detect your target in the 700 nm channel.

   Note: *It is recommended to use 700 nm channel detection for your most abundant target and the 800 nm channel for weak or low abundance targets.*

3. Image the membrane in the 700 and 800 nm channels with an Odyssey® Imaging System. If saturation occurs, reduce the scan intensity or acquisition time, or use AutoScan if your instrument includes this.

   Note: *After destaining and Western blot processing, 1-3% residual fluorescence from Revert may be seen during imaging in the 700 nm channel, but this will not impact results.*

Method 3: Total Protein Detection After Western Blot Detection

This method is useful if total protein staining is desired, but not performed on the membrane prior to Western blot detection.

You will image the membrane two times in this protocol, once in step 2 and once in step 5. The first acquisition will be of the target proteins. The second acquisition will be of the Revert 700 Total Protein Stain.

Note: *This method* ***only*** *works if the membrane is processed with a protein-free blocking buffer and antibody diluent.*

Step 1: Process Western Blot with Protein-Free Antibody Diluent and Blocking Buffer

Block membrane in Intercept® Protein-Free Blocking Buffer and use Intercept Protein-Free Antibody Diluent for primary and secondary antibody incubations.

Step 2: Acquire Image for Target Channel(s)

Image membrane to capture detection channels for target proteins.

Step 3: Rinse Membrane with Ultrapure Water

Rinse the membrane with ultrapure water.

Note: *Before moving to the next step, ensure the membrane container provides a minimum clearance of 1/8th of an inch on all sides. Revert 700 Total Protein Stain will cause the membrane to swell. Without clearance, staining may be uneven.*

Step 4: Stain with Revert 700 Total Protein Stain

1. Add methanol to the stain reagents as indicated on each bottle.
2. Stain the membrane with Revert 700 Total Protein Stain. Incubate the membrane in 10 mL of Revert 700 Total Protein Stain solution for 5 minutes at room temperature with gentle shaking.

   Note: *Do not allow the membrane to dry from this point on.*
3. Decant the total protein stain solution thoroughly. Using approximately 10 mL of Revert Wash Solution for each wash, rinse the membrane two times for 30 seconds at room temperature with gentle shaking.
4. Decant the wash solution thoroughly, then briefly rinse the membrane with ultrapure water.

Step 5: Acquire Revert 700 Total Protein Stain Image

Note: *Do not allow the membrane to dry during imaging. If you are using an Odyssey® DLx or an Odyssey® M, it is best to place the silicone mat on top of the membrane. See the Operator's Manual for your imager for detailed instructions (licor.com/support).*

Immediately image the membrane in the 700 nm channel using the Odyssey® M Imager.

Western Blot Analysis Using Empiria Studio® Software

Empiria Studio Software is designed for reliable analysis of near-infrared Western blots using publisher guidelines. Create a new experiment in Empiria Studio, then choose the appropriate workflow: Linear Range Validation with Total Protein Stain or Target Analysis with Total Protein Stain. Follow the steps provided in the workflow.

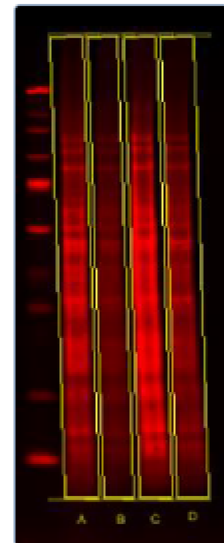
Empiria Studio uses workflows to minimize user-to-user variation, provides more extensive analysis options, and can compute vital statistical values. To get started with Empiria Studio, visit licor.com/empiria-support.

Western Blot Analysis Using Image Studio™ Software

Use Image Studio Software for quick signal quantification.

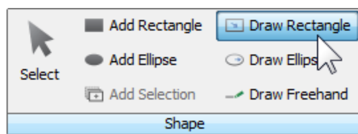
Total Protein Quantification Using Image Studio™ Software

Before you begin, under the **Analysis** tab change the type to **Manual.** Use the **Draw Rectangle** tool in Image Studio Software to quantify the total protein signal in each lane.



1. Draw a shape around the first lane on the total protein stain image (700 nm channel image).

   a. In the **Shape** group of the **Analysis** tab, click **Draw Rectangle**.

   b. Draw a rectangle around the first lane.

   If lanes are skewed, rotate the shape by clicking **Rotate** in the **Edit** group.

   More Info: *For help choosing the right background subtraction method, see licor.com/BgSubtractHelp.*

2. Add the shape from the first lane to each remaining lane.

   a. With the first shape selected, click **Add Selection**.

   b. Click the next lane to copy the shape.

   If necessary, adjust the shape borders to ensure that each shape encloses the signal from one, and only one lane.

3. Export total protein quantification data.

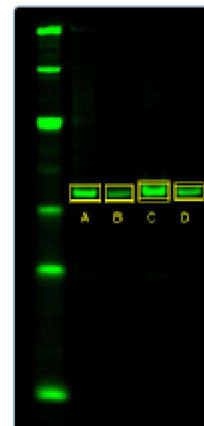
   a. Click **Shapes** to open the Shapes data table.

   b. Select shape data, then copy and paste data into a spreadsheet, or click **Report** to export the data into an external spreadsheet.

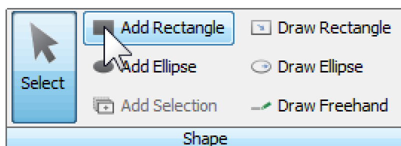
   Note: *All data fields will be exported, but "Signal" is the field of interest for analysis.*

Target Protein Quantification Using Image Studio™ Software

Before you begin, under the **Analysis** tab change the type to **Manual.** Use the **Add Rectangle** tool in Image Studio Software to quantify the target bands.



1. Click to select your Western blot target channel image, then add shapes to bands.
    a. In the **Shape** group of the **Analysis** tab, click **Add Rectangle**.



    b. Click each band to be analyzed, and an appropriately sized shape will be added around the band.

    More Info: *For help choosing the right background subtraction method, see licor.com/BgSubtractHelp.*

2. Export quantification data.
    a. Click **Shapes** to open the Shapes data table.

    b. Select shape data, then copy and paste data into a spreadsheet, or click **Report** to export the data into an external spreadsheet.

    Note: *All data fields will be exported, but "Signal" is the field of interest for analysis.*

Normalization Calculation

1. Open the spreadsheet containing total protein and target quantification values.
2. Calculate the Lane Normalization factor for signal data from the lanes on the Total Protein Stain image.

$$\text{Lane Normalization Factor} = \frac{\text{TPS for Each Lane}}{\text{TPS Signal from the Lane with the Highest TPS Signal}}$$

Channel	Lane Name	Signal	Highest Signal	Lane Normalization Factor
700	A	900	1000	0.9
700	B	700	1000	0.7
700	C	1000	1000	1
700	D	800	1000	0.8

Example numbers shown for illustrative purposes only.

3. Calculate the normalized signal for each band by dividing the signal for each band by the Lane Normalization Factor for the lane the band is in.

$$\text{Normalized Signal} = \frac{\text{Target Band Signal}}{\text{Lane Normalization Factor}}$$

Channel	Lane Name	Signal	Lane Normalization Factor	Normalized Signal
800	A	90	0.9	100
800	B	70	0.7	100
800	C	100	1	100
800	D	80	0.8	100

Example numbers shown for illustrative purposes only.

4. Use the **Normalized Signal** for quantitative comparisons.

Related Products

926-11011	Revert™ 700 Total Protein Stain
927-60001	Intercept® (TBS) Blocking Buffer
927-70001	Intercept (PBS) Blocking Buffer
927-80001	Intercept (TBS) Protein-Free Blocking Buffer
927-90001	Intercept (PBS) Protein-Free Blocking Buffer
927-65001	Intercept T20 (TBS) Antibody Diluent
927-75001	Intercept T20 (PBS) Antibody Diluent
927-85001	Intercept T20 (TBS) Protein-Free Antibody Diluent
927-95001	Intercept T20 (PBS) Protein-Free Antibody Diluent
928-60000	Chameleon® Duo Pre-stained Protein Ladder (for visual and two-color near-infrared detection)
926-31090	Odyssey® Nitrocellulose Membranes
928-40004	4X Protein Sample Loading Buffer (optimized for use with near-infrared detection)

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