# Advan Stain™ Total Fluorescent Protein Staining Kits

Rapid fluorescent stain for total protein normalization



AdvanStain Total is a fluorescent protein stain for PVDF and Nitrocellulose membranes that allows for sensitive and quantitative visualization of protein bands. For precise quantitative Western blot data, it is critical to perform normalization to account for sample inconsistencies, sample loading errors and uneven transfer. In an effort to increase transparency, total protein normalization is quickly becoming the standard for Western blot normalization as it is now the preferred method for top tier journals. The AdvanStain Total Fluorescent Protein Staining Kits provide a convenient method for total protein normalization that is compatible with digital imaging systems capable of detecting Cy3 and other optically similar dyes.



# AdvanStain<sup>™</sup> Total Fluorescent Protein Staining Kits

Rapid fluorescent stain for total protein normalization

### Advantages

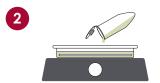
- **CONVENIENT** simple protocol that takes less than 20 minutes, no de-staining required
- VERSATILE compatible with chemiluminescent and IR700/IR800 fluorescent Western blotting
- ACCURATE linear across a broad range from 1–50µg of lysate, ideal for total protein normalization



### AdvanStain Total Fluorescent Protein Staining Kits workflow

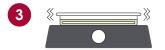


After electrotransfer is complete, transfer blot to incubation tray filled with water Incubate membrane for 5 minutes at RT with gentle agitation.



#### Decant water and dispense 5–10mL of AdvanStain Total Staining Solution into incubation tray

Incubate PVDF membranes for 5 minutes and Nitrocellulose membranes for 10 minutes at RT with gentle agitation.



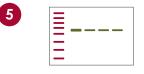
#### Wash membrane

3X for 3 minutes with 5–10mL Washing Solution with gentle agitation.



## Image blot for total protein analysis

PVDF membranes may be imaged wet or dry using green-light excitation. Nitrocellulose membranes must be imaged wet.



#### Prepare for Western blotting

If the PVDF membrane is imaged wet, rinse blot with water before proceeding with Western blot. If imaged dry, pre-wet the blot with 100% Methanol then rinse with water before proceeding with Western blot.



# AdvanStain<sup>TM</sup> Total Fluorescent Protein Staining Kits

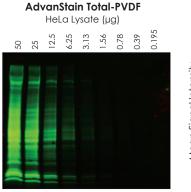
Rapid fluorescent stain for total protein normalization

### AdvanStain Total spectrum Broad linear range

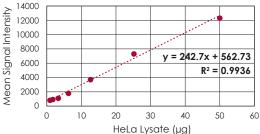
AdvanStain Total in 1X PBS

#### Normalized excitation 1.0 - Normalized emission Ex<sub>max</sub> 515nm Fluorescence (RFU) Em<sub>max</sub> 536nm 0.5 0.0 700 300 400 500 600 Wavelength (nm)

Figure 1. AdvanStain Total can be imaged with a CCD or laser based imaging system using green-light excitation.



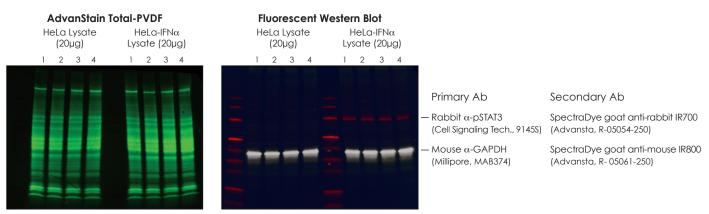
#### **Scatter Plot and Linear Regression**



Blot imaged in Cy3 channel

Figure 2. AdvanStain Total has a broad linear range from  $1-50\mu g$  of lysate with an  $R^2$ value of 0.99 for the linear regression fit, ideal for protein normalization.

## Compatible with two-color IR fluorescent Western blotting



Blot imaged in Cy3 channel

IR700 (red) and IR800 (gray-scale) channels

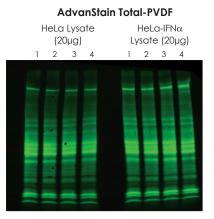
Figure 3. AdvanStain Total is compatible with IR fluorescent Western blotting, without destaining. Both high and low abundance proteins are easily detected in the IR700 and IR800 channels after AdvanStain Total membrane staining without destaining.



# AdvanStain<sup>™</sup> Total Fluorescent Protein Staining Kits

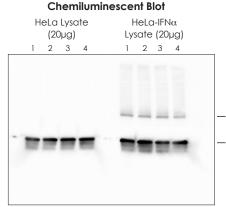
Rapid fluorescent stain for total protein normalization

### Compatible with chemiluminescent Western blotting



Blot imaged in Cy3 channel

## Maximum flexibility



Blot developed with WesternBright ECL HRP Substrate(Advansta, K-12045-D50) Rabbit α-pSTAT3 (Cell Signaling Tech., 9145S) Rabbit α-Actin (Millipore, MABT523) Figure 4. AdvanStain Total is compatible with chemiluminescent Western blotting without de-staining. Both high and low abundance proteins are easily detected after AdvanStain Total membrane staining

without destaining.

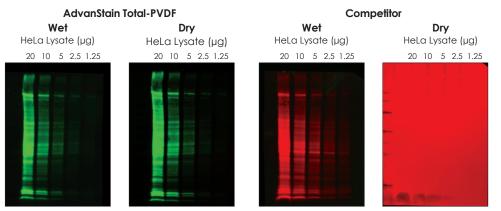


Figure 5. AdvanStain Total may be imaged wet or dry without increasing general background.

### Ordering Information

Catalog Number	Product	Size	
K-11061-015	AdvanStain™ Total-PVDF Fluorescent Protein Staining Kit	15 blots	
K-11062-015	AdvanStain™ Total-NC Fluorescent Protein Staining Kit	15 blots	

#### Advansta Corporation

2140 Bering Drive | San Jose, CA 95131 Tel: 650.325.1980 | Fax: 650.325.1904 | Email: sales@advansta.com Product information: www.advansta.com/products/AdvanStain-Total Copyright © 2021 Advansta. All rights reserved. The Advansta logo and WesternBright<sup>®</sup> are registered trademarks of Advansta Corporation. AdvanStain<sup>™</sup> is the trademark of the Company. All other trademarks, service marks and tradenames appearing in this brochure are the property of their respective owners.

