

# WesternDot® Secondary Antibody Conjugates

Pub. no. MAN0009672

Revision B.0

Detailed protocol is available on our website. Go to [www.lifetechnologies.com](http://www.lifetechnologies.com) and search for the product number.

## Usage guidelines

- For best signal-to-background, use nitrocellulose membranes.
- Avoid Tween® 20-containing buffers, particularly when using PVDF membranes.
- You can use non-fat milk (5% w/v solution) instead of casein (sodium salt). We do not recommend BSA for blocking or WesternDot® conjugate incubation steps.
- Some primary antibodies (e.g. anti-phosphoprotein) may be incompatible with casein. See detailed protocol for more information.
- Start with the manufacturers recommendation for primary antibody concentrations.
- When imaging blots, orient the membrane with the sample-side facing the excitation light source. For a transmitted UV source, this means placing the blot sample-side face down.

For Research Use Only. Not for use in diagnostic procedures.

## Quick Protocol for Single Protein Detection

**Note:** For multiplex detection, refer to the detailed protocol available online.

1. **Prepare Solution 1:** Dissolve 1 g casein (sodium salt) per 50 mL of PBS or TBS (2% w/v solution).
2. Block membrane with 10 mL of **Solution 1** for 1 hour.
3. Incubate membrane in primary antibody diluted in **Solution 1** at 4°C overnight.
4. **Prepare Solution 2:** Add 20 µL of WesternDot® reagent to 10 mL of **Solution 1** (1:500 dilution).
5. Wash the membrane 3× with PBS, then incubate in **Solution 2** at room temperature for 1 hour.
6. Wash the membrane 3× with PBS, rinse with water, air-dry, and image.

### Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at [www.lifetechnologies.com/termsandconditions](http://www.lifetechnologies.com/termsandconditions). If you have any questions, please contact Life Technologies at [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

**DISCLAIMER** - LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

©2014 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Tween is a registered trademark of Uniqema Americas, LLC.

**For support visit** [lifetechnologies.com/support](http://lifetechnologies.com/support)  
[lifetechnologies.com](http://lifetechnologies.com)