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Datasheet for KFA017

DyLight™ Multiplex 649/549 Duo Western Blot Kit

Overview

Description:	DyLight™ Multiplex 649/549 Duo Western Blot Kit - KFA017
Item No.:	KFA017
Size:	1 Kit
Applications:	WB

Product Details

Background:	The DyLight™ multiplex 649/549 Duo fluorescent Western blotting kit is suited for simultaneous detection and quantification of specific protein populations in a biological sample. Using a combination of two antibodies selected for minimal cross reactivity, fluorescent detection method enables simultaneous quantitative analysis of multiple proteins within the same sample on the same blot. The DyLight™ multiplex 649/549 Duo Western blot kit contain all the necessary components that are optimized for the simultaneous detection of multiple proteins on the same blot using DyLight™-dye labeled secondary antibodies that are visualized in different fluorescence channels (649/549). The kit also includes blocking buffer, wash buffer, pre-stained protein standard and an incubation box for convenience and ultimate performance with minimal or no optimization. The fluorescent dyes such as DyLights™ when conjugated to secondary antibodies, offer a variety of benefits over traditional detection methods such as colorimetric and chemiluminescent detection. Multiplex detection using the correct lighting and filter conditions, enables the quantitation of multiple proteins and eliminates the need to strip and reprobe. Other benefits of fluorescent Western blotting include increased sensitivity, excellent signal stability over time as well as precise quantitative analysis with broader dynamic range and high linearity. Due to their exceptional photostability, DyLight™ dye conjugates can be archived and visualized several times without a decrease in signal.
Synonyms:	Fluorescent western blotting, Multiplex western blotting, multi-color western blotting, fluorescent labelled antibodies, fluorescent imaging, DyLights, dyLight conjugates
Detection Kit Type:	Fluorescent Western Blot Kit

Target Details

Purity/Specificity:	The DyLight conjugated secondary antibodies were prepared from monospecific antiserum by immunoaffinity chromatography using Rabbit or Mouse IgG coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Goat Serum, Rabbit or

	Mouse IgG, and Rabbit or Mouse Serum. No reaction was observed against Bovine, Chicken, Goat, Guinea Pig, Hamster, Horse, Human, Rat and Sheep Serum Proteins. These antibodies will react with heavy chains of rabbit or mouse IgG and with light chains of most rabbit or mouse immunoglobulins. Blocking buffer is specifically formulated to achieve superior reproducible western blotting images using this system. Wash buffer (10X TTBS) was aseptically filtered through a Millipore 0.22 micron filter into clean, pre-sterilized containers. The product was tested on trypticase soy agar for 24 hours, 48 hours and 72 hours and was found to be negative for bacteria.
Relevant Links:	• DyLight™ Multiplex Flourescent Western Blot Protocol

Application Details

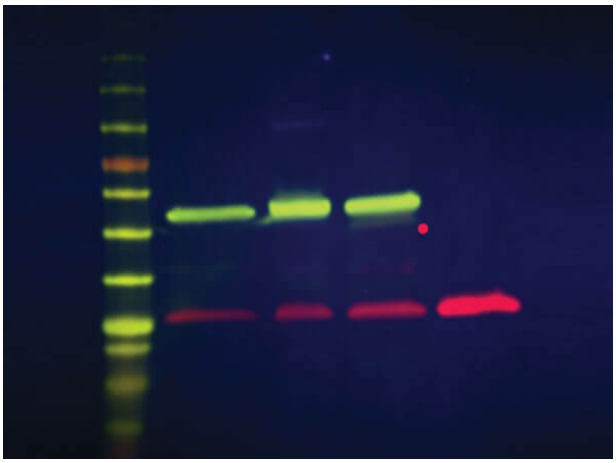
Tested Applications:	WB
Application Note:	This DyLight™ Multiplex 649/549 Duo Western Blot Kit contains: Goat anti-Mouse IgG (H+L) DyLight™ 549; Goat anti-Rabbit IgG (H+L) DyLight™ 649; Opal pre-stained protein standard; Wash buffer (10X TTBS); Blocking buffer (2x) and incubation box. This kit is suitable for fluorescent western blotting, multiplex analysis, including multicolor imaging, utilizing various commercial gel imaging systems.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.

Formulation

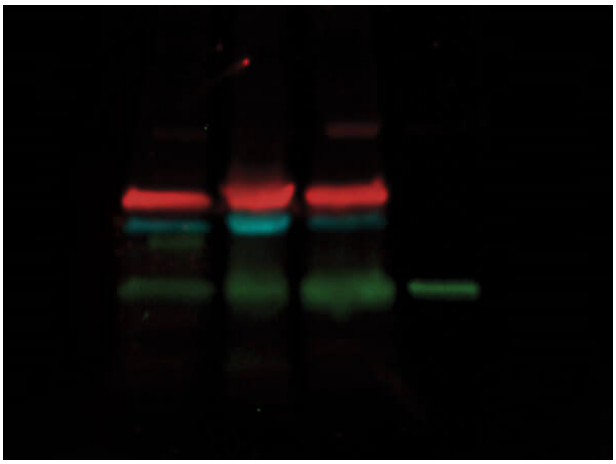
Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	The secondary antibodies should be stored at 4°C prior to reconstitution. For extended storage aliquot contents and freeze at -20°C. Avoid freeze/thaw cycles. The wash buffer and blocking buffer can be stored at 2-8°C prior to opening. The pre-stained protein Western standards should be stored at 2-8°C. The pre-stained protein standards can also be stored at room temperature for up to 6 months or at -20°C for up to two years.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Western Blot
Simultaneous detection of α-tubulin and GFP on a single blot using Rockland DyLight™-labeled secondary antibody conjugates. Protein lysates from HeLa (lane 1), PC12 (lane 2) and K562 (lane 3) cells and 100ng GFP protein (Lane 4) were run on a gel. The cell lysates were spiked with 25ng, 50ng and 75ng GFP protein. Probing of cell lysates and GFP with mouse anti-α-tubulin and chicken anti-GFP antibodies followed by DyLight™ 649 goat anti-mouse IgG (pseudocolored green) and DyLight™ 800 goat anti-chicken IgG (red) conjugates, and then imaged using Syngene G:BOX Imaging System resulted in comparable patterns of detection. Lane 5: Rockland Opal Prestained Protein Standard 10-245kDa.



Western Blot
Simultaneous detection of three proteins on a single blot using Rockland DyLight™-labeled secondary antibody conjugates. Protein lysates from HeLa (Lane 1), PC12 (Lane 2) and K562 (lane 3) cells and 50ng GFP protein (Lane 4) were run on a gel. The cell lysates were spiked with 50ng, 75ng and 150ng GFP protein. Probing of cell lysates and GFP with anti-α-tubulin (mouse), anti-β-actin (rabbit), and anti-GFP (chicken) followed by DyLight™ 649 goat anti-mouse IgG (red), DyLight™ 800 goat anti-rabbit IgG (pseudocolored aqua) and DyLight™ 488 goat anti-chicken IgG (pseudocolored green) conjugates, and imaged using Syngene G:BOX Imaging System resulted in comparable patterns of detection.

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.