WesternDot™ 625 Western Blot Kits

Catalog nos. W10132 and W10142

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
WesternDot™ blocking buffer (Component A)	320 mL	1X solution	• 2–6˚C • DO NOT FREEZE	When stored as directed these kits are stable for 6 months
Wash buffer (Component B, 0.5 M Tris-HCl, 1.5 M NaCl, 0.5% Tween 20, pH 7.4)	320 mL	10X concentrate		
Biotin-XX goat anti-mouse IgG (H+L) (Component C, for W10132, Goat Anti-Mouse Kit) **OR** Biotin-XX goat anti-rabbit IgG (H+L) (Component C, for W10142, Goat Anti-Rabbit Kit)	80 µL	2 mg/mL		
Qdot® 625 streptavidin conjugate, (Component D)	80 µL	1 µM solution in 1 M betaine, 50 mM borate pH 8.3 with 0.05% sodium azide		
WesternDot™ staining dish (Component E)	2 each	Not applicable		
Number of reactions: Sufficient material is supplied for 20 mini blots (8 cm × 8 cm) using the protocol described below.				
Approximate fluorescence excitation/emission maxima: Excitation: 254 to 488 nm; emission maximum: 625 nm, see Figure 2.				

Introduction

The WesternDot™ 625 Western Blot Kits combine the unique properties of Qdot® 625 nanocrystals with the high affinity streptavidin-biotin interaction to allow simple, highly sensitive detection of proteins on western blots. The WesternDot™ Kits contain optimized, ready-to-use or ready-to-dilute reagents for sensitive immunodetection of proteins immobilized on nitrocellulose (NC) or polyvinylidene difluoride (PVDF) membranes.

Incorporating a standard western blotting protocol, the detection step relies on a biotinylated secondary antibody, goat anti-mouse (Cat. no. W10132) or goat anti-rabbit (Cat. no. W10142) followed by the key component, a Qdot® 625 streptavidin conjugate (Figure 1). The extremely high extinction coefficient of the Qdot® 625 nanocrystal in the UV and blue wavelengths combined with high quantum yield and an orange/red emission (Figure 2) allow for sub-nanogram sensitivity of protein detection using standard UV or blue-light based detection systems. The fluorescent signal is compatible with the commonly used modes of fluorescent detection of DNA or protein gels and does not require specialized emission filters (Table 2).

The WesternDot™ kits include a specially formulated WesternDot™ blocking buffer, Wash buffer, and two staining dishes that hold sufficient solution volumes recommended for a standard mini-blot (8 cm × 8 cm). The kits include sufficient reagents for 20 mini-blots.

Qdot® 625 Streptavidin Conjugate

The Qdot® 625 streptavidin conjugate is made from a nanometer-scale crystal of a semiconductor material (CdSe), which is coated with an additional semiconductor shell (ZnS) to improve the optical properties of the material. The core-shell material is further coated with a polymer shell that allows the materials to be conjugated to biological molecules and retain their optical properties. This polymer shell is directly coupled to streptavidin through an active ester coupling reaction yielding a material with streptavidin covalently attached on the surface (typically 5–10 streptavidins/Qdot® conjugate), which results in Qdot® streptavidin conjugates with high specific biological activity. The Qdot® 625 streptavidin conjugate is the size of a large macromolecule or protein (~15–20 nm). The Qdot® 625 streptavidin conjugate has a narrow, symmetric emission spectrum with the emission maximum near 625 nm. For details on the optical properties, spectral characteristics, and biological activity of Qdot® 625 streptavidin conjugate, refer to the manual supplied with Qdot® 625 streptavidin conjugate available separately from Invitrogen (Cat. no. A10196).

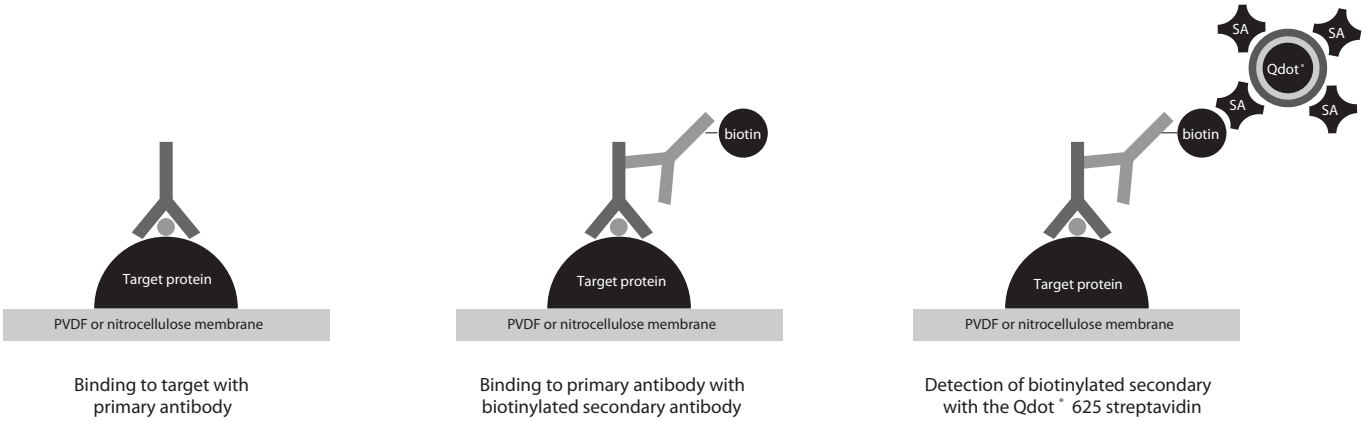

Figure 1. Schematic for the WesternDot™ kit.

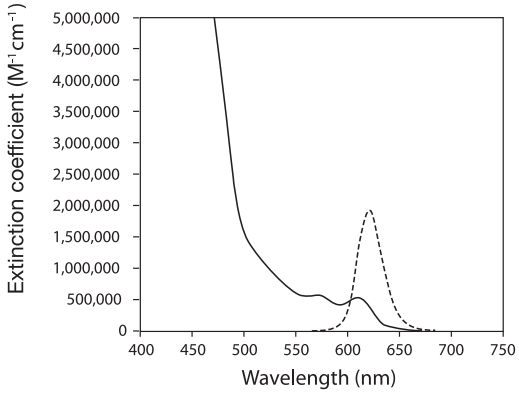

Figure 2. Spectral properties of the Qdot® 625 streptavidin conjugate.

Before You Begin

Materials Required but Not Provided

- Blotted PVDF or nitrocellulose transfer membranes containing applied antigen (**Note:** We have obtained best results with low background fluorescence PVDF membranes such as Immobilon®-FL PVDF transfer membrane from Millipore.)
- Primary antibody to detect applied antigen (visit www.invitrogen.com/antibodies for a list of antibodies available from Invitrogen)
- Ultra pure water
- Orbital shaker or rocking platform
- Forceps for manipulating blotted membranes
- UV transilluminator/ethidium bromide filter/Polaroid camera or Imaging system (see Table 2 for a list of compatible instruments)

Preparing 1X Wash Buffer

Prepare 1X Wash Buffer for each blot by combining 16 mL 10X Wash Buffer (Component B) with 144 mL ultra pure water. The resulting 1X Wash Buffer contains 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween®-20.

Disposal of Qdot® Conjugate

The Qdot® conjugate contains cadmium and selenium in an inorganic, crystalline form. Dispose of the material in compliance with all applicable local, state, and federal regulations. For more information on the composition of these materials, consult the Material Safety Data Sheet.

Caution

The WesternDot™ blocking buffer (Component A) contains 0.1% sodium azide. Wear gloves when handling this solution. Harmful if swallowed. In case of accident or if you feel unwell, seek medical advice immediately. Dispose of this material and its container as hazardous waste.

Experimental Protocols

General Guidelines

To obtain the best results with WesternDot™ Kits:

- Perform all steps on an orbital (rotary) shaker or rocking platform (rotating at 1 revolution/second). Be sure that the solutions cover the membrane and move freely over and around it.
- Process the blot face up (but still submerged) for good fluid flow.
- Avoid touching the working surface of the membrane, even with gloves.
- Work quickly when changing solutions as PVDF membranes dry quickly. If the membrane dries, re-wet the membrane with methanol and rinse with water before proceeding.
- Add solutions to the trays slowly, at the membrane edge, to avoid bubbles forming under the membrane. Decant from the same corner of the dish to ensure complete removal of previous solutions.

Preparing the Membrane

Immediately after transferring the proteins to a nitrocellulose or PVDF membrane, wash the membrane twice for 5 minutes with 20 mL water to remove gel and transfer buffer components. Proceed to **Immunodetection Protocol**.

If you are using water-washed and dried nitrocellulose membranes, proceed to **Immunodetection Protocol**. If you are using water-washed and dried PVDF membranes, re-wet the membrane in 100% methanol briefly for 30 seconds, followed by two 20 mL water washes for 5 minutes, and then proceed to **Immunodetection Protocol**.

Immunodetection Protocol

Perform all steps at room temperature with continuous shaking using an orbital shaker (set at ~ 1 revolution/second) or with continuous rocking for the indicated times.

1.1 Place the membrane in WesternDot™ staining dish (Component E) containing 8 mL WesternDot™ blocking buffer (Component A). Incubate for 60 minutes on a shaker set at 1 revolution/second.

Note: Blocking step may be performed overnight at 4°C.

1.2 Prepare 8 mL of the diluted primary antibody in 1X wash buffer at the appropriate concentration.

Note: Primary antibody concentration depends on the manufacturer's recommendation, and is typically 0.1 to 2.0 µg/mL.

1.3 Decant the blocking buffer and add diluted primary antibody solution from Step 1.2 to the blot. Incubate for 60 minutes on a shaker. Decant solution.

Note: Primary antibody incubation may be as short as 30 minutes or overnight at 4°C.

1.4 Wash the membrane for 5 minutes with 15 mL 1X Wash buffer, then decant. Repeat wash step 2 more times.

1.5 During the last wash, prepare the secondary antibody solution by diluting 4 µL Biotin-XX-Goat anti-rabbit or Biotin-XX-Goat anti-mouse (Component C) into 8 mL 1X Wash buffer.

1.6 Decant wash buffer and incubate the membrane in 8 mL secondary antibody solution from Step 1.5 for 30–60 minutes, then decant.

1.7 Wash the membrane for 5 minutes with 15 mL 1X Wash buffer, then decant. Repeat wash step 2 more times.

1.8 During the last wash, prepare Qdot® 625 streptavidin conjugate by diluting 4 µL Qdot® 625 streptavidin conjugate (Component D) into 8 mL WesternDot™ blocking buffer (Component A).

1.9 Decant wash buffer and incubate the membrane in 8 mL Qdot® 625 streptavidin conjugate solution from Step 1.8 for 30–60 minutes, then decant.

Note: Incubation with Qdot® 625 streptavidin conjugate may be as short as 15 minutes or as long as several hours to overnight. For a strong antibody and abundant antigen, signal may be visible within 5 to 10 minutes when the blot is examined *in situ* with a hand-held UV lamp.

1.10 Wash the membrane for 5 minutes with 15 mL 1X Wash buffer, then decant. Repeat wash step 2 more times. Perform a final wash with 15 to 20 mL ultra pure water.

Note: The blot may be stored in ultra pure water, 1X Wash buffer, TBS (Tris-buffered saline), or PBS (phosphate buffered saline) overnight with minimal signal loss.

Imaging the Blot

PVDF or Immobilon®-FL membranes

For best results with PVDF membranes, dry the membrane and image with epi-illumination.

- With epi-illumination, image the membrane **wet or dry** using exposures ranging from 2 seconds to 2 minutes.
- With trans-illumination using a UV trans-illuminator, image the membrane **wet** using exposures ranging from a few milliseconds to several seconds.

Nitrocellulose membranes

- With UV epi-illumination, image the membrane **wet or dry** using exposures ranging from 2 seconds to 2 minutes.
- With trans-illumination, membranes can be imaged **wet or dry**. There will be 3- to 5-fold lower sensitivity and increased background with trans-illumination as compared to epi-illumination.

Instrumentation

See Table 2 for a list of compatible instruments.

Excitation

Deep blue or UV light in trans- or epi-illumination.

Lasers with 473 nm or 488 nm excitation are acceptable.

Emission filters

Filters that are qualified for ethidium bromide, SYPRO® Ruby, SYPRO® Red, Qdot® 605, Qdot® 625, or Qdot® 655 are suitable. Filters centered around 625 nm provide greater signal. Most orange-to-red emission filters are also acceptable.

Signal stability

Under the reaction conditions, Qdot® nanocrystals are chemically stable and photophysically stable. Repeated images may be taken over the course of several hours and the signal lasts for several days to months, though there may be a loss in sensitivity at the lower end. If a membrane begins to dry, re-wet the membrane as described in **Preparing the Membrane**. If using dried membranes, image with epi-illumination for better results.

Examples of Expected Results

Examples of expected results using the WesternDot™ 625 Western Blot Kits are shown in Figures 3 and 4.

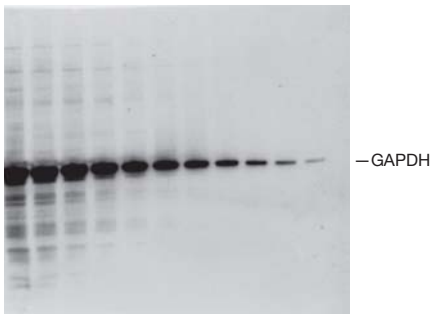

Figure 3. Example of results using the WesternDot™ 625 Kit with a PVDF membrane: Total proteins (2-fold dilution series ranging from 10 µg to ~10 ng) from Jurkat cell extract were analyzed on a NuPAGE® Novex® 4–12% Bis-Tris gel and then transferred to an Immobilon®-FL PVDF membrane. Immunodetection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an endogenous "housekeeping" protein in the Jurkat cell extract was performed with WesternDot™ 625 Goat Anti-Mouse Western Blot Kit (Cat. no. W10132) using a mouse monoclonal anti-GAPDH antibody (Invitrogen Cat. no. 39-8600) at 1 µg/mL as described in this manual. The wet membrane was imaged using an Alpha Innotech HD2 instrument with a SYPRO® Red emission filter (620 nm ±40 nm) and excitation at 302 nm trans-illumination with an exposure time of 300 milliseconds.

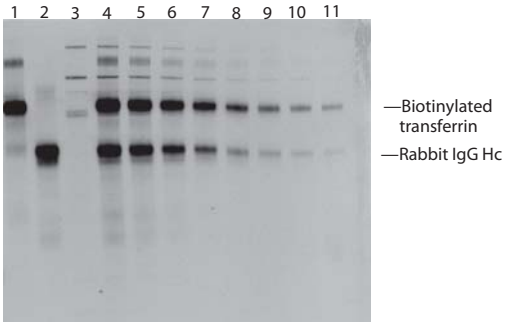

Figure 4. Example of results using the WesternDot™ 625 Kit with a nitrocellulose membrane: Jurkat cell extract was spiked with biotin-XX- transferrin (Invitrogen Cat. no. T23363) and rabbit IgG, and was analyzed on a NuPAGE® Novex® 4–12% Bis-Tris gel. Proteins were transferred to nitrocellulose membrane using the iBlot™ Gel Transfer System. Immunodetection of biotin-XX- transferrin and rabbit IgG in the Jurkat cell extract was performed with WesternDot™ 625 Goat Anti-Rabbit Western Blot Kit (Cat. no. W10142) as described in this manual. The membrane was imaged using an Alpha Innotech HD2 instrument with a SYPRO® Red emission filter (620 nm ±40 nm) and excitation at 254 nm epi-illumination with an exposure time of 45 seconds.
The biotin-XX-transferrin is directly detected using the Qdot® 625 streptavidin conjugate (Component D) while biotinylated secondary" antibody (Component C) serves as a primary antibody for rabbit IgG detection and illustrates the utility of the Qdot® 625 streptavidin conjugate for detection via any biotinylated primary antibody. Biotinylated proteins that are intrinsic to the extract (see lane 3) will be detected with the WesternDot™ Kits.
Lane 1: 1 ng biotin-XX- transferrin; Lane 2: 10 ng rabbit IgG; Lane 3: 5 mg Jurkat whole-cell extract; Lane 4: 5 mg Jurkat whole-cell extract spiked with 10 ng rabbit IgG and 1 ng biotin-XX- transferrin; Lanes 5-11: 2-fold dilution series of the sample applied to Lane 4.

Table 2. Imaging platforms recommended for detecting signal using WesternDot™ 625 Western Blot Kits.

Manufacturer	Instrument
Alpha Innotech	AlphaDigiDoc PRO
	AlphaImager® EP
	AlphaImager® HP
	FluorChem® HD2
BioRad	ChemiDoc XRS
	GelDoc XR
	PharosFX Systems
	VersaDoc MP 4000
Fuji Film Life Sciences	FLA-7000
	FLA-8000
	FLA-9000
	LAS-3000
	LAS-4000
GE Healthcare	Typhoon™
	Storm™ with Blue LED
Invitrogen	Safe Imager™
Kodak	Gel Logic
	Image Station 4000 MM and PRO
	Image Station 4000R and PRO
Syngene	DigiGenius
	G: Box fluorescence and chemiluminescence
	InGenius
	U: Genius
UVP	Benchtop UV Transilluminators
	BioDocIt™ Imaging System
	DigiDocIt
	EC3™ Imaging System
	FirstLight® UV illuminator
	Visi-Blue™ Transilluminator

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
W10132	WesternDot™ 625 Goat Anti-Mouse Western Blot Kit *20 minigel blots*	1 kit
W10142	WesternDot™ 625 Goat Anti-Rabbit Western Blot Kit *20 minigel blots*	1 kit
Related Products		
A10196	Qdot® 625 streptavidin conjugate *1 µM solution*	200 µL
B2763	Biotin-XX goat anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
B2770	Biotin-XX goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
IB1001	iBlot™ Gel Transfer Device	1 each
IB3010-02	iBlot™ Transfer Stack, Mini (Nitrocellulose)	10 sets/box
LC5800	Novex® Sharp Pre-stained Protein Standard	2 × 250 µL
LC5925	SeeBlue® Plus2 Pre-Stained Standard	500 µL
S37102	Safe Imager™ Blue Light Transilluminator	1 each

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