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Technical **Data Sheet**

iD Western 1H
ID-WBYYYY-XXX

Eurogentec products are sold for research or laboratory use only and are not to be administered to humans or used for medical diagnostics.

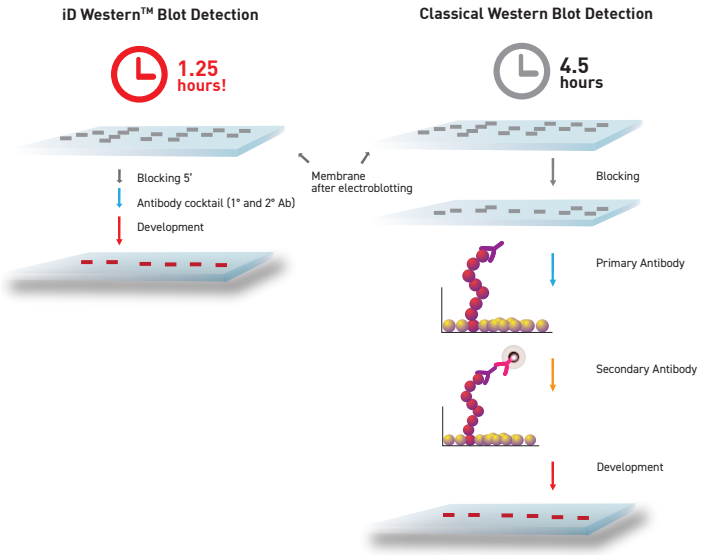
Products

Cat #	Description	Size
ID-WBETR1-005	Compatible with a rabbit primary antibody and a colorimetric detection	1 Kit– 5 assays
ID-WBEHR1-005	Compatible with a rabbit primary antibody and a chemiluminescent detection	
ID-WBSHR1-005	Compatible with a rabbit primary antibody and a sensitive chemiluminescent detection	
ID-WBETM1-005	Compatible with a mouse primary antibody and a colorimetric detection	
ID-WBEHM1-005	Compatible with a mouse primary antibody and a chemiluminescent detection	
ID-WBSHM1-005	Compatible with a mouse primary antibody and a sensitive chemiluminescent detection	

Description

iD Western 1H kits yield publication-quality Western or Dot blot results in about one hour. This kit replaces the classical 3-step Western process, which takes nearly 5 hours.
The iD Western 1H Kits are designed to produce high signal with low background for quick and clear Western analysis of proteins.

The kits contain all the necessary reagents, buffers and materials for performing a Western blot, and no additional secondary antibody is needed.



Key Features

- FAST Process
- Easy to perform
- Low background
- Reproducible results
- No additional secondary antibody needed

Storage

Store the iD Nitrocellulose Membrane at room temperature. Store the rest of the kit at 4°C. It will remain stable for two years. Do not freeze the kit or any of its components.

Kit Content

Components	Enhanced Kits		Sensitive Kits
	With TMB	With HRP	
Pretreat Solution A	50 mL	50 mL	50 mL
Pretreat Solution B	50 mL	50 mL	50 mL
WB-1 Solution	0.5 mL	0.5 mL	0.5 mL
WB-2 Solution	50 mL	50 mL	50 mL
5x Wash Solution	125 mL	125 mL	125 mL
iD Nitrocellulose Membrane (ID-WCNIM1-005)	5 sheets	5 sheets	5 sheets
iD TMB Substrate	15 mL	-	-
iD HRP Substrate	-	2x 7.5 mL	-
iD HRP Super Chemoluminescent Substrate	-	-	2x 7.5mL

Protocol

This procedure is optimized for a sheet of 7.5 x 8.0 cm membrane. However, reagent volumes can be scaled up or down according to the size of the membrane used.

Reagents not provided

Purified primary antibodies: Affinity-purified antibodies are recommended. Further optimization may be needed if the serum containing the antibody is to be used.

1X Wash Solution Preparation

Dilute 25 mL of 5X wash solution with 100 mL of distilled or filtered water to make 125 mL of 1X wash solution. If any precipitate forms in the 5X wash solution during storage, incubate the bottle in a warm or hot water bath (up to 50°C) with occasional mixing until all the precipitate disappears.
Use 15 mL of 1X wash solution for each rinse and 20 mL of 1X wash solution for each wash.

Mixture 1 Preparation

Before or during protein transfer, prepare Mixture 1:
– Mix the primary antibody with WB-1 in a microcentrifuge tube
– Vortex Mixture 1 gently for a few seconds
– Centrifuge briefly
– Incubate Mixture 1 at room temperature for at least 40 minutes.

	Enhanced kit with TMB	Enhanced kit with HRP	Sensitive kit
Mixture 1	ID-WBETR1-005 ID-WBETM1-005	ID-WBEHR1-005 ID-WBEHM1-005	ID-WBSHR1-005 ID-WBSHM1-005
WB-1 Solution	50 – 100 µL	20 – 100 µL	5 – 25 µL
Primary Antibody*	5 – 10 µg	2 – 10 µg	0.5 – 2.5 µg
Ratio of WB-1: Antibody	10 µL: 1 µg	10 µL: 1µg	10 µL: 1 µg
For Antibody without known Titer	Mix 5 µg of Ab with 50 µL WB-1	Mix 5 µg of Ab with 50 µL WB-1	Mix 1 µg of Ab with 10 µL WB-1

*Refer to manufacturer's recommendations for the appropriate amounts of antibody. With iD Western 1H - Sensitive Kits, use 1/4 to 1/2 of the recommended amount. For antibodies without known titers, start with 1 µg for iD Sensitive Kits and 5 µg for other iD Western 1H Kits.

Membrane

– Just before the protein transfer from gel to membrane is completed, mix 10 mL of Pretreat Solution A with 10 mL of Pretreat Solution B in a plastic container to make the blocking solution mixture.

➲ *Always prepare and use fresh solution mixture.*

– Place the membrane directly in the blocking solution mixture
– Incubate on a shaker for five minutes at room temperature.
– After incubation, rinse the membrane twice with 15 mL of 1X wash solution.

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Incubation with antibody solution

– Add Mixture 1 to 10 mL of WB-2 in a Western blot box and mix well.
– Incubate the membrane in this solution (WB-2 containing Mixture 1) on a shaker at room temperature for 40 minutes.

This solution (WB-2 containing Mixture 1) may be recovered and reused up to three times if stored at 4°C. However, this may cause variations to arise due to changes in antibody concentration and carryover contamination.

– Rinse the membrane once with 15 mL of 1X wash solution.
– Wash the membrane on a shaker three times for ten minutes each with 20 mL of 1X wash solution.

When using the TMB substrate, wash the membrane three times for just five minutes each with 20 mL of 1X wash solution.

Use a clean container for each wash step to avoid carryover contamination and to reduce background.

Signal Development with Chemiluminescent HRP Substrate

– Prepare the Working Solution
Mix 1.5 mL of Reagent A with 1.5 mL of Reagent B by vortexing for a few seconds to make the working solution.

Working Solution Preparation

Reagent	**Volume**
Reagent A	1.5 mL
Reagent B	1.5 mL
Total Volume	3.0 mL

0.05 mL of the working solution is sufficient to cover 1 cm^2 of membrane. When protected from light, the working solution (A+B) remains stable for several hours at room temperature.

– Drain the excess wash solution from the membrane by holding the membrane vertically with forceps and touching the edge against a tissue.
– Place the membrane on a clean, flat surface, and cover the membrane with working solution.
– Incubate for 3 minutes at room temperature.
– Place the membrane on a soft, clean tissue.
– Use another tissue to remove excess working solution.
– Wrap the membrane in a clean piece of plastic film.
– Expose to a sheet of film (not provided) for 30 seconds and then develop. Repeat with different exposure time if necessary. An imager capable of detecting chemiluminescent signals can also be used to record the results.

Signal Development with colorimetric HRP (TMB) Substrate

iD TMB Substrate is a ready-to-use working solution, and 0.05 mL is sufficient to cover 1 cm^2 of membrane.

– Drain the excess wash solution from the membrane by holding the membrane vertically with forceps and touching the edge against a tissue.
– Place the membrane on a clean plate and cover it with TMB.
– Incubate for 5 to 10 minutes at room temperature until the desired color intensity is reached.
– Stop the reaction by rinsing the membrane three times for thirty seconds each in 20 mL of deionized water.
– Drain off the excess water and transfer the membrane to a piece of paper towel.
– Air-dry the membrane in a dark place.

For further information please contact our Customer Help Desk:

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