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Technical **Data Sheet**

iD HRP Substrate Kit
ID-SUHRP1-060

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

Description
iD HRP Substrate Kit detects horseradish peroxidase (HRP) on immunoblots such as Western and Dot blots. The reagents are optimized for high sensitivity, high intensity, long duration, and low background.

Key Features
- Fast & Easy procedure, only a 3-minute incubation period.
- High sensitivity and low background
- Reproducible results

Storage
Store the kit at 2 - 8°C. It will remain stable for 12 months.

Kit Content
The kit contains two reagents for HRP signal development, reagent A and reagent B.

Components	ID-SUHRP1-060
Reagent A	30 mL
Reagent B	30 mL
Enough Reagents for	10 mini gel (7.5 x 8 cm) Western or Dot blot detections, or 600 cm² of membrane*

* based on 0.1 ml of the working solution per cm²

Protocol
This kit is designed for the development of membranes from Western and Dot blots after the final wash.

1. Mix one volume of reagent A with one volume of reagent B by vortexing for a few seconds. This forms the working solution.

   ◘ *Use 0.1 ml of the working solution per cm² of membrane. The working solution should be warmed up to room temperature before use. The working solution is stable for several hours at room temperature when protected from light.*

2. Drain the excess wash solution from the membrane by holding the membrane vertically with forceps and touching its edge against a tissue.
3. Place the membrane on a clean, flat surface, and cover the membrane with the working solution.
4. Incubate for 3 minutes at room temperature.
5. Place the membrane on a clean tissue.
6. Use a soft clean tissue to remove excess working solution.
7. Wrap the membrane in a clean piece of plastic film.
8. In a dark room, expose the membrane to a sheet of film for 20 seconds
9. Develop the exposed film.

Repeat this step with different exposure times if required.

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Troubleshooting

Problem	Probable cause	Solution
The signal is weak or invisible.	There is too little protein loaded.	Load more protein(s) onto the SDS-PAGE gel
	Poor transfer efficiency	Optimize the transfer time and/or the electrical current
	The incubation time is too short or the reagent is not warm enough.	The working solution should be warmed up to room temperature before use. Increase the incubation time to five minutes to increase the signal intensity.
There is high background or non-specific bands on the blot	The wash time is too short.	Add an additional wash step with 1X wash solution always decreases background.
	The signal development time is too long.	Reduce the development time
	The reagents or equipment have been contaminated.	Use a clean container every time you change the solution before washing. Wear gloves and use clean forceps to handle the membranes.
	There is too much working solution.	Remove excess working solution using a soft clean tissue

For further information please contact our Customer Help Desk:

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