Instructions for use

Signal Enhancer Western-ELISA – Set, Sol. 1 and Sol. 2

For immunochemistry.
For enhancement of immunochemical detection signals on blot membranes and in ELISA.
Enhancer solution

- Strong enhancement of signal strength
- Most easy application
- Compatible with
  - Western and ELISA
  - HRP and AP
  - all common membranes
  - all common substrates

The **Signal Enhancer Western/ELISA** enhances antigen-antibody reactions in a variety of immunoassays such as Western blotting, dot blotting and ELISA. It can significantly enhance detection of weak immunoreactive and low abundance target proteins and is well compatible with all common assay versions, membranes and ELISA plates. Signal enhancement is dependent on several parameters such as the particular antigen/antibody pair, the particular detection enzyme, the substrate chosen and so on. In most cases, however, the signal strength is about 5 to 10fold higher than with the substrate alone.
Signal Enhancer Western/ELISA**-1** is recommended for enhancement of reactions between antigens and their corresponding **primary antibodies**. In most cases, the signal strength is about 5fold higher than with the substrate alone.
Signal Enhancer Western/ELISA**-2** is recommended for enhancement of the binding of **secondary antibodies**. In most cases, the signal strength is about 5fold higher than with the substrate alone. Also recommended for performance of assays using **one antibody only**, which is **labelled**.

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Content
The kit contains Signal Enhancer Western/ELISA-1 (Art. No. 9213) and Signal Enhancer Western/ELISA-2 (Art. No. 9214).
1 Mini-Set: 2 x 50 ml (for approx. 5 mini gel blots), 1 Set: 2 x 250 ml (for approx. 25 mini gel blots).
One bottle with 250 ml of Signal Enhancer 1 or 2 is sufficient for approx. 25 assays.

Application for Western- and Dot Blotting
Note: For all steps, use sufficient volumes to completely immerse the blot.
Preliminary: Following gel electrophoresis, ransfer proteins onto a nitrocellulose or PVDF membrane. For dot blotting, spot proteins directly onto a membrane.
RT = room temperature

1. Block the membrane with a suitable blocking reagent. If only one (labelled) antibody is used, skip steps 2 to 4 and proceed with step 5.
2. Meanwhile dilute the primary antibody (ratio as established) with Signal Enhancer Western/ELISA-1.
3. Incubate the membrane in this primary antibody solution at RT for one hour under slight agitation.
4. Wash three times with TBS-T or PBST.
5. Meanwhile dilute secondary antibody with Signal Enhancer Western/ELISA-2 (ratio as established).
6. Incubate the membrane in this solution prepared in step 2 at RT for one hour under slight agitation.
7. Wash three times with TBS-T or PBST.
8. Continue with an appropriate detection procedure to detect the protein of interest.

Application for ELISA (sandwich)

Preliminary: Prepare a 96-well ELISA plate with solid-phase antibodies.
RT = room temperature

1. Block the wells with a suitable blocking reagent.
2. Dilute the *antigen* with Signal Enhancer Western/ELISA-1.
3. Dilute *primary antibody* with Signal Enhancer Western/ELISA-1 (ratio as established).
4. Add antigen sol. and primary antibody sol. into each well and incubate at 37 °C for one hour.
5. Wash with PBST three times.
6. Meanwhile dilute secondary antibody with Signal Enhancer Western/ELISA-2.
7. Add secondary antibody to each well and incubate at 37 °C for one hour.
8. Wash with PBST three times.
9. Continue with an appropriate detection procedure to detect the protein of interest.

Trouble Shooting:

Western-/Dot Blotting		
Prob-lem	**Possible Cause**	**Solution**
Weak signal	Low protein concentration after electrophoresis	Use samples of as high concentration as possible in electrophoresis. Serial dilution of protein is useful in determining optimal concentration.
	Insufficient transfer to membrane	Increase electric current or transfer time. Check buffer compostion. Usually the higher the gel concentration the lower the transfer efficiency. In transfer of gradient gels, the difference in transfer efficiency between high-molecular weight and low-molecular weight proteins is increased. Efficiency may be improved by switching from semi-dry to wet transfer.
Western-/Dot Blotting		

Prob-lem	Possible Cause	Solution
Weak signal	Membrane transfer time too long and/or electric current too high	If using nitrocellulose membrane, excessive transfer can cause protein to permeate across the membrane to the opposite side. Reduce electric current or shorten time, make sure methanol is present during transfer. Optionally: change to PVDF membrane. If using PVDF membrane, make sure SDS is present during the transfer.
	Low antibody concentration	Determine optimal antibody concentration by serial dilution.
Too many extra bands	Antibody concentration too high	Excessive antibody can increase nonspecific signals. Determine optimal antibody concentration by serial dilution.
	Antigen concentration too high	Apply less concentrated protein in electrophoresis. Serial dilution is useful in determining optimal concentration.
	Insufficient blocking	Check the type and concentration of the blocking agent used, try other blocking reagents.
	Insufficient washing	Increase frequency of washing.

Western-/Dot Blotting		
Prob-lem	**Possible Cause**	**Solution**
Colour-less band center	Antibody concentration too high / signals ‚flashed'	Depending on the detection reagent used, optimal luminescence may be hindered by excessive signals ('flashed'), since substrate is used up too quickly / before film exposure. Reduce amount of secondary antibody / determine optimal concentration by serial dilution.

ELISA		
Problem	**Possible Cause**	**Solution**
Weak signals	Antigen or antibody concentration not high enough	Optimize antigen and antibody concentrations by serial titration.
Colour too intense	Antigen or antibody concentration too high	Optimize antigen and antibody concentrations by serial titration.
	Duration of exposure too long	Shorten exposure time.
High back-ground signals	Antigen or antibody concentration too high	Optimize antigen and antibody concentrations using serial titration.
	Insufficient blocking	Check the type and concentration of the blocking agent used, try other blocking reagents.
	Insufficient washing	Increase frequency of washing.

ELISA		
Problem	**Possible Cause**	**Solution**
Great variance in reading	Problem with the ELISA plate	Protein binding efficiency can vary greatly among different types of ELISA plate or among different batches of the same type of ELISA plate. When more accurate measurement is needed, select the ELISA plate carefully.

Further Recommended Products:
ROTI®Block, protein-free (Art. No. A151)
ROTI®DAB-Kit (Art. No. 9202)
DAB, powdered (Art. No. CN75)
DAB Metal Enhancer (Art. No. 9204)
ROTI®Stock 10x PBS (Art. No. 1058)
ROTI®Stock 10x PBST (Art. No. 1059)
ROTI®Stock 10x TBS (Art. No. 1060)
ROTI®Stock 10x TBST (Art. No. 1061)
Hydrogen peroxide 30 % (Art. No. 9681)
ROTI®Lumin plus (Art. No. 3692).

Storage: Storage temp.: +4 °C,
Transport temp.: cooled

Signal Enhancer Western/ELISA

9211.2	Mini-Set
9211.1	Set
9213.1	250 ml Enhancer Western/ELISA-1
9214.1	250 ml Enhancer Western/ELISA-2