HisDirect Western Blot Staining Pads for Direct Detection of His-Epitope Tagged Proteins

Product Information

Catalog No.

HisDirect-PA-010 (10x) pmol/fmol-Range

HisDirect-PA-010 (30x) pmol/fmol-Range

Detection Antibody:	Clone 13/45/31-2
Isotype:	Mouse IgG1, kappa
Specificity:	Recognizes N-terminal, C-terminal or internal HIS-tagged fusion proteins with at least 6 histidine residues
Immunogen:	Recombinant (HIS)6-p53 protein
Species Reactivity:	Not applicable
Conjugation:	60 nm **n**anogold particles (Amax=540 nm)
Presentation:	Antibody conjugated to 60 nm gold nanoparticles in PBS with 1-2% BSA and 0,35% Tween 20

Application

Western Blot, Dot-Blot

Microbial Colony / Plate Test

(nitrocellulose / PVDF membranes)

Transport Temperature:	ambient (4 – 40 °C)
Storage Temperature:	room temperature (18 – 23 °C)

General recommendations, optimal dilutions should be determined by the end user by titration test

Recommended blocking buffer (not provided):

5 % Sucrose in TBS with 0,1 % Tween

Product Description

HisDirect is a conjugate of mouse monoclonal anti-(His)6-tag antibody, clone 13/45/31-2 (H. Zentgraf/DKFZ Heidelberg, Germany) to 60nm-nano gold particles that can be used to directly detect His-Tag proteins. The HisDirect protocol combines antibody binding, washing and detection in one step and is suitable for Western Blotting and Dot-Blot on nitrocellulose and PVDF membranes and can also be used in microbial colony and plate tests. The protocol with an incubation time of 10 – 60 minutes **is a one step protocol were no** additional washing steps, detection reagent or substrate incubation is required.

The **HisDirect** staining result is a pinkish colored precipitation directly on the membrane that can be visually analyzed and documented with any stadard camera. One **HisDirect Pad** contains enough conjugate to stain one 8 x 10cm Minigel when resolved in 10 ml of blocking buffer. The sensitivity can be adusted by resolving 1 – 3 pads in 10ml buffer:

Sensitivity – ready to use Solution

Quantitative Detection Range		10 kDa	30 kDa	50 kDa	100 kDa
1 Pad / 10ml	WB Mini Gel	0,24 - 1,5 µg	0,72 - 4,5 µg	1,2 - 7,5 µg	2,4 - 15 µg
Pmol-Range (8-50 pmol/mm^2)	Dot Blot (0,5µl)	0,08 - 0,5 µg	0,24 - 1,5 µg	0,4 - 2,5 µg	0,8 - 5 µg
2 – 3 Pads / 10 ml	WB Mini Gel	2,4 - 150 ng	7,2 - 450 ng	12 - 750 ng	24 - 1500 ng
Fmol-Range (80 - 1000fmol/mm^2)	Dot Blot (0,5µl)	0,8 - 50 ng	2,4 - 150 ng	4 - 250 ng	8 - 500 µg

Short Protocol

- Transfer His-tagged protein to nitrocellulose or PVDF membranes
- Wash membrane with A. dest.
- Transfer membrane to a clean container
- Add 10 ml of blocking buffer* and 1 – 3 Pads
- Incubate for 10 to 90 min on a shaker
- After incubation dry membrane between filters papers
- Incubation in HisDirect Solution can be prolonged in order to increase sensitivity
- Document results with any commercially available camera

* Recommended blocking buffer: 5 % Sucrose in TBS with 0,1 % Tween (not provided)

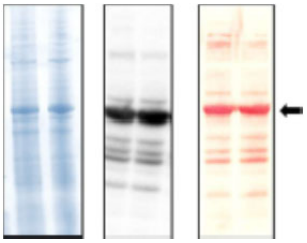

Fig.1: Comparison of conventional detection methods and HisDirect reagent using lysates of two High Five® insect cell culture expression approaches. The arrow denotes the target protein. Left: Coomassie staining (3 h); Middle: HRP-anti-Penta-His antibody with ECL reagent (4 h); Right: HisDirect (1 Pad) (20 minutes)

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Detailed Protocol

HisDirect Pads contain anti-His-Tag antibody conjugated to gold nanoparticles bound to a tissue pad. In order to resuspend the conjugate, place 1-3 pads in 10 ml blocking buffer (not provided) together with the membrane. The shelf life for Pads is at least 12 month from date of receipt. The shelf life can be extended if the results are carefully monitored using appropriate positive and negative controls.

Additional Material Required: Incubation container, filter paper, laboratory shaker, camera
Blocking Buffer: 5 % Sucrose in TBS with 0,1 % Tween.

1. Transfer His-tagged protein to nitrocellulose or PVDF membranes (Western Blot / Dot Blot).
2. Let the membrane dry to fix the proteins. If necessary this process can be accelerated by using a non-heating blow dryer.
3. Rinse the membrane thoroughly in A. dest.

   THIS STEP IS ESSENTIAL IN ORDER TO REMOVE ANY DENATURING AGENTS THAT MAY INHIBIT THE REACTION OF THE TAGGED PROTEIN TO THE HIS-DIRECT REAGENT!
4. Place the membrane (max 10x10cm) in a clean container and cover with 10 ml of blocking buffer.
5. Add 1 - 3 pads to the blocking buffer, according to the required sensitivity.
6. Incubate the membrane on a laboratory shaker for 10 - 90 minutes until the desired staining intensity is reached. Normally an incubation time of 10-30 minutes is sufficient. The intensity of the signal directly correlates with the number of bindings sites of the antibody.
7. Dry membrane on a filter paper before analyzing and documenting the results. For documentation any commercial camera can be used. Quantification should be performed using an accompanying standard curve and color intensity matching (see example below)
8. In case of an insufficient staining result step 5 - 6 can be repeated. • Wash membrane with A. dest.

Example of quantification of results

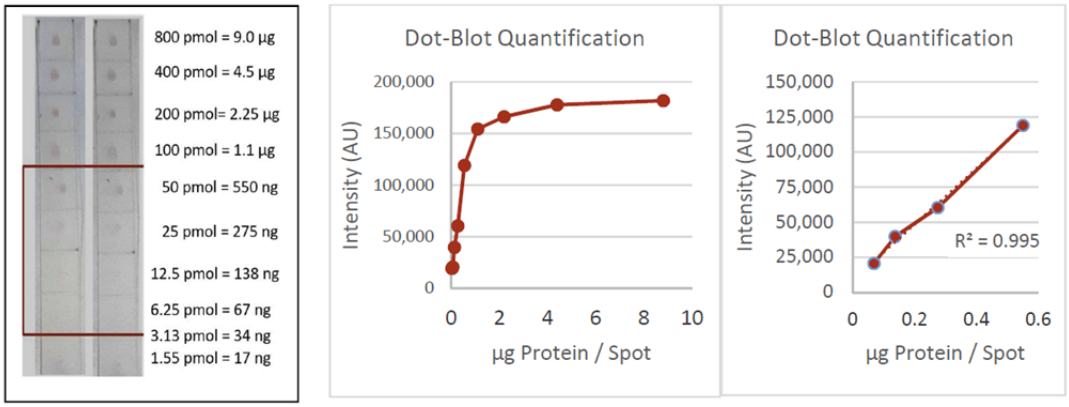

Fig.2 Quantification of poly-histidine labelled protein using HisDirect reagent. Left: Dot blot for the quantitative determination of His6-labelled 11 kDa proteins. The red frame marks the linear detection range. Left: On a nitrocellulose strip, 0,5 µL each of a protein dilution series were incubated for 90 min in 10 mL HisDirect reagent, dried and photographed, the photo converted into greyscale, and the amount of protein applied noted; Middle: Quantification with the aid of a dilution series. Correlation between signal intensity and the amount of protein applied determined from the greyscale image using the quantification program Image Studio Lite 5.2. Right: The linear range lies between a protein amount of 6,35 pmol and 50 pmol, or 69 ng and 0,55 µg, for areas of approx. 1mm^2.

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