

# HisDirect ready-to-use Western Blot Staining Solution for Direct Detection of His-Epitope Tagged Proteins

## Product Information

### Catalog No.

HisDirect-LP-010 (10x10ml) pmol-Range (ready-to-use)  
HisDirect-LF-010 (10x10ml) fmol-Range (ready-to-use)

### Presentation:

Antibody conjugated to 60 nm  
Gold Nanoparticles in PBS with 1-  
2% BSA and 0,35% Tween 20

### 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 nanogold particles  
(Amax=540 nm)

### 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

## 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** where no additional washing steps, detection reagent or substrate incubation is required.

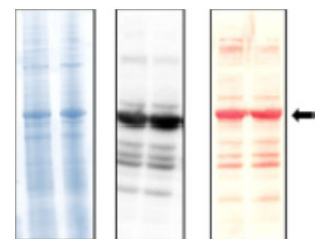
The **HisDirect** staining result is a pinkish colored precipitation directly on the membrane that can be visually analyzed and documented with any standard camera. **HisDirect** Reagent is available as ready to use reagent in two sensitivity ranges the **pmol-Range** (overexpression) and the **fmol-Range** (moderate expression / interaction studies).

## Sensitivity – ready to use Solution

Quantitative Detection Range		10 kDa	30 kDa	50 kDa	100 kDa
<b>Pmol-Range</b> ( 8-50 pmol/mm <sup>2</sup> )	WB Mini Gel	0,24 - 1,5 µg	0,72 - 4,5 µg	1,2 - 7,5 µg	2,4 - 15 µg
	Dot Blot (0,5µl)	0,08 - 0,5 µg	0,24 - 1,5 µg	0,4 - 2,5 µg	0,8 - 5 µg
<b>Fmol-Range</b> (80 - 1000fmol/mm <sup>2</sup> )	WB Mini Gel	2,4 - 150 ng	7,2 - 450 ng	12 - 750 ng	24 - 1500 ng
	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
- Cover membrane with HisDirect ready-to-use solution
- Incubate for 10 to 90 min
- 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



**Fig.1:** Comparison of conventional detection methods and HisDirect ready-to-use 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 ready-to-use Pmol-Range (20 minutes)

For in vitro research use only. Not for diagnostic or therapeutic use.



## Detailed Protocol

HisDirect Ready-to-use reagent contains anti-His-Tag antibody conjugated to gold nanoparticles. The solution does not contain any preservatives and should be used directly after opening the vial. The shelf life of the unopened vial is at least 6 months from date of receipt. The shelf life can be extended if the results are carefully monitored using appropriate positive and negative controls.

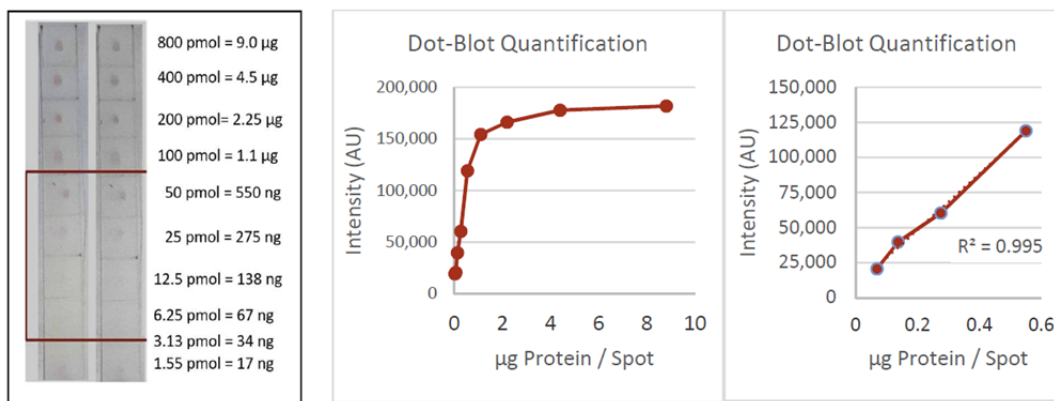
**Additional Material Required:** Incubation container, filter paper, laboratory shaker, camera

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. Carefully invert the HisDirect ready-to-use Reagent in order to resuspend any settled conjugate.
5. Place the membrane in a clean container and cover the membrane with HisDirect Ready-to-use reagent. No previous blocking step is required (10 ml ready-to-use reagent should be used for a membrane of max. 10x10cm)
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 binding 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 ready-to-use 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 ready-to-use 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<sup>2</sup>.

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