Product Information

Catalog No. RabbitDirect-L-005
RabbitDirect-L-010 (5x10ml) fmol-Range (ready-to-use)

Detection Antibody:	AffiniPure Goat Anti-Rabbit IgG
Isotype:	IgG
Specificity:	Fc Fragment Specific
Species Reactivity:	Rabbit Fc, may cross-react with immunoglobulins from other species
Conjugation:	gold nanoparticles (Amax=540 nm)
Presentation:	Antibody conjugated to gold nanoparticles in assay dilution

Application

Western Blot, Dot-Blot,
ELISA, EM, Microbial Colony / Plate Test
(nitrocellulose and PVDF membranes)

Transport Temperature:	ambient (8 – 30 °C)
Storage Temperature:	room temperature (15 – 25 °C)

Primary Antibody: 2 ug / 10 mL reagent, optimal dilutions should be determined by the end user by titration test

Product Description

Rabbit-Direct is a conjugate of goat polyclonal anti-rabbit Fc antibody to gold nanoparticles. It can be used to directly detect aby protein with its specific antibody, if derived from rabbit. The **Direct** protocol combines antibody binding, washing and detection in one step. It is suitable for Western Blot and Dot-Blot immonostainings on nitrocellulose and PVDF membranes. The **Direct** reagent can also be used in membrane based microbial colony and plate tests. Additionally the reagent is suitable for ELISA assays and even microscopy sampling, like EM or dark field. The staining protocol with an incubation time of 60 minutes is a **single step protocol** were no additional washing steps, detection reagent or substrate incubation is required.

The **Direct** staining result is a red colored accumulation of the gold label on the membrane. The immunostaining thus gives a visible read out. It is easily documented with any standard camera or gel documentation system (settings: ponceau red). The **Direct** reagent is activated by addition of 2 ug primary antibody into a tube containing 10 mL reagent. The mixture is simply poured onto the priviously blocked membrane carrying the protein and or the sample. The membrane is than incubated on an orbital shaker for about one hour. Access reagent is rinsed of with tap water and the membrande is dried for enhancing the signal to noise ratio. Prolonged staining will enhance the signal without risking bluring.

Sensitivity – ready to use solution mixed with primary antibody

The sensitivity of the Direct reagent-based immunoassay is primarily dependent on the primary antibody's sensitivity. Thus, for critical detection ranges in the low femto mole range, optimization of primary antibody is recommended. The gold-nanoparticles give a visible signal in the sub-femto mole range.

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Short Protocol

• Transfer protein onto a nitrocellulose or PVDF membrane

• Transfer membrane to a clean container

• Block remaining protein binding sites with 1% BSA in 1x PBS or in 1 x TBS for 5 minutes

• Add 2 ug (2uL 1 mg/mL) suitable primary antibody into one vial of 10 mL

Direct ready-to-use reagent solution. Mix carefully but thoroughly by inverting five times.

• Incubate for 1 hour.

• Take out the membrane, briefly rinse off access reagent with tap water.

• Dry the membrane on filter paper.

• Assess the result visually.

• Document results with any commercially available camera or gel doc system.

• If the signal intensity should be intensified, you may prolong the incubation.

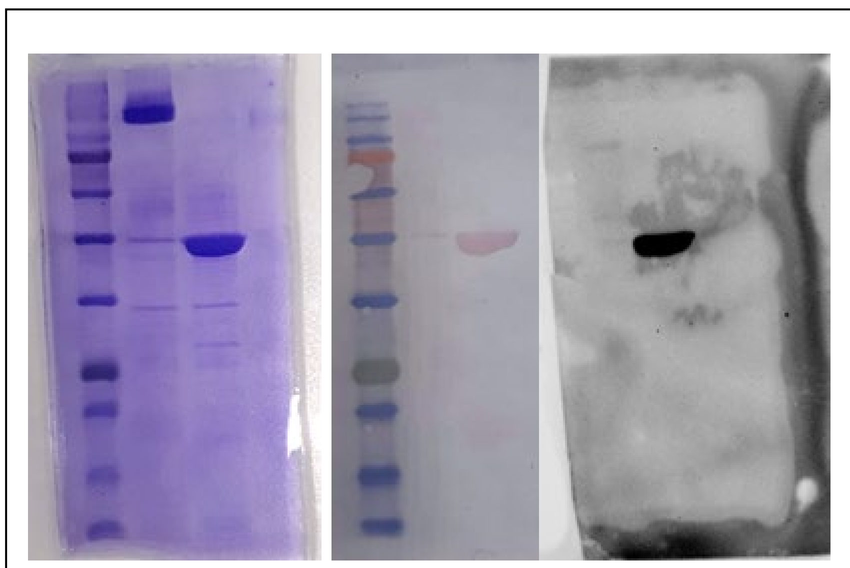

Fig.1: Example of H6-TolC test expression analyzed by SDS-PAGE, Coomassie blue stained (left), Mouse-Direct with DIA-900 mouse anti-His-Epitope antibody (middle) and ECL-stained anti-His-HRP antibody (SantaCruz) (right).

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Detailed Protocol

Rabbit-Direct ready-to-use reagent contains anti-rabbit secondary 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 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 tray, filter paper, orbital shaker, camera

1. Transfer the protein of interest onto nitrocellulose or PVDF membrane (Western Blot / Dot Blot).

2. Place the membrane in a clean container and block remaining protein binding sites with 1% BSA in 1x PBS or in 1 x TBS for 5 minutes. This step additionally washes of remaining detergent from the electrophoresis and transfer steps.

   Any minor amount of cross-contamination might disturb the accuracy of the reaction. Ideally use singe use tray, like large weighing boats.

3. **Add 2 ug (2 µL 1 mg/mL) suitable primary antibody produced in rabbit to one vial containing 10 ml Rabbit-Direct reagent. Carefully invert the vial several times, allowing the primary antibody to dilute evenly.**

   If the expected protein range is in the low femto mole range, optimization of the primary antibodies' quantity might be required.

   If unknown primary antibodies or older stocks are to be used, it is highly recommended to test the antibodies performance in a dot blot assay using just 1 mL Direct reagent in a 3 mL weighing boat for incubation. Parallel setups are suitable to compare sensitivity, specificity and cross-reactivity.

4. Discard the blocking solution and cover the membrane with the **Direct** ready-to-use reagent mixture. 10 ml ready-to-use reagent should be used for a membrane of max. 10 x 10 cm size.

5. Incubate the membrane on a laboratory orbital shaker for one hour. Or at least until the desired staining intensity is reached. At saturation the intensity of the signal directly correlates to the number of antibody binding sites.

6. Take out the membrane and rinse of access reagent with tap water.

7. Dry the membrane on filter paper before analyzing and documenting the results. For documentation any commercial camera can be used. Alternatively, documentation can be done using a gel documentation system, selecting the Ponceau Red settings. 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 - 7 can be repeated.

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Example of quantification of results

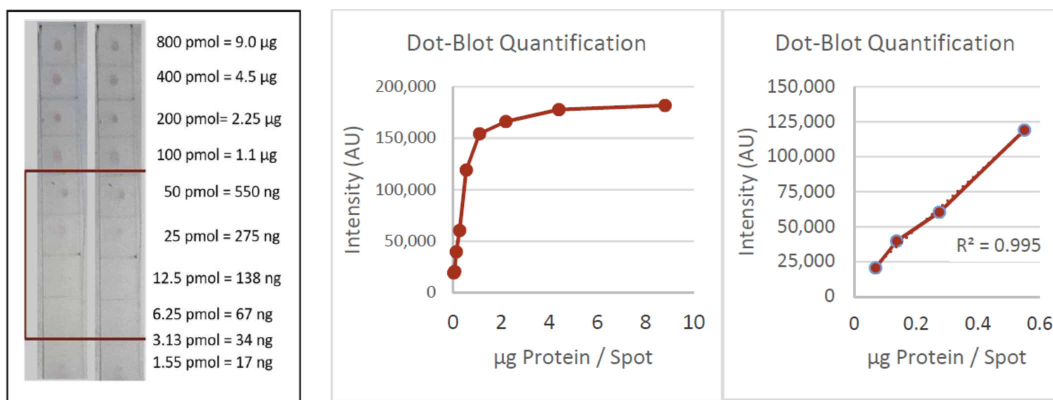

Fig.2 Quantification of poly-histidine labelled protein using HisDirect ready-to-use Reagent. Left: Dot blot for the quantitative determination of His6-labelled 11 kDa proteins. The red frame marks the linear detection range. Left: On a nitrocellulose strip, 0,5 µL each of a protein dilution series were 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^2.

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