Product Datasheet

PIA-PINK-His Ready-to-Use Reagent

Gold labelled mouse-anti-(His)6-tag antibody for immunodetection

Product number and packaging unit

Figure 1: PIA-PINK-His ready-to-use reagent

 # V102-12 PIA-PINK-His pico ready-to-use reagent 10 Pc. of 10 mL (10 applications)

 # V102-11 PIA-PINK-His femto ready-to-use reagent 10 Pc. of 10 mL (10 applications)

Overview

Product name	**PIA-PINK-His ready-to-use reagent**
Specificity	Reacts with recombinant proteins containing an epitope of at least 6 histidine residues (N-, C-terminus or both termini). A higher number of histidine residues leads to an increase in binding affinity.
Immunogen	Recombinant (HIS) 6-p53 protein
Host species	Mouse
Class	Monoclonal
Clone	Clone 13/45/31-2
Isotype	Mouse IgG1, kappa
Conjugation	60 nm gold nanoparticles with an absorption maximum of 540 nm
Tested applications	Western blot, dot blot
Other applications	Colony test, plate test
Product form	Mouse IgG1 conjugated to gold nanoparticles (⌀ 60 nm) in 10 ml T/PBS pH 7.4 supplemented with BSA, Tween 20/Triton X-100. The antibody was purified from the cell culture supernatant.
Transport	Ambient temperature (4 °C - 40 °C)
Storage conditions	Room temperature (18 °C - 23 °C)
Shelf life	Unopened 6 months

PIA-PINK-His is only for research and development purposes. Not approved for medical applications.

PIA-PINK-*His* ready-to-use reagent is a conjugate of anti-His-tag antibody and gold nanoparticles. It is suitable for immuno-based detection of poly-histidine-labelled proteins.

PIA-PINK-*His* ready-to-use reagent is tested for western blot and dot blot. The reagent can also be used for detection in colony tests and plate tests. It reduces the steps of antibody binding, washing and detection to a single step, usually completed in 10 to 60 minutes. No secondary antibody is needed.

PIA-PINK-*His* ready-to-use reagent is supplied in liquid form in 10 mL aliquots. Each aliquot contains the optimal amount for staining an 8 x 10 cm mini-gel.

On the blot membranes, the result is visible as a persistent, pink-coloured accumulation of the conjugate on the target protein, and can be documented with commercially available cameras.

The detection range of quantitative analysis can be varied by selecting the appropriate product series.

The quantitative detection range of the *pico* **products** is in the pico-mol range. The *pico* series is suitable for blot detection of proteins from overexpression approaches.

The quantitative detection range of the *femto* **products** is in the femto-mol range. The *femto* series is suitable for blot detection of proteins from moderate expression approaches and interaction studies.

The following table gives guideline values for the detection range of poly-histidine-labelled proteins of different molecular weights.

Table 1: Quantitative Detection Ranges

Dot Blot 0.5 µL drops	**10 kDa**	**30 kDa**	**50 kDa**	**100 kDa**
Pico 8 - 50 pmol/mm^2	0.08 – 0.5 µg	0.24 – 1.5 µg	0.4 – 2.5 µg	0.8 – 5 µg
Femto 80 – 5,000 fmol/mm^2	0.8 – 50 ng	2.4 - 150 ng	4 – 250 ng	8 – 500 ng

Western Blot of Mini-Gels	**10 kDa**	**30 kDa**	**50 kDa**	**100 kDa**
Pico 8 - 50 pmol/mm^2	0.24 – 1.5 µg	0.72 – 4.5 µg	1.2 – 7.5 µg	2.4 – 15 µg
Femto 80 – 5,000 fmol/mm^2	2.4 – 150 ng	7.2 - 450 ng	12 – 750 ng	24 – 1500 ng

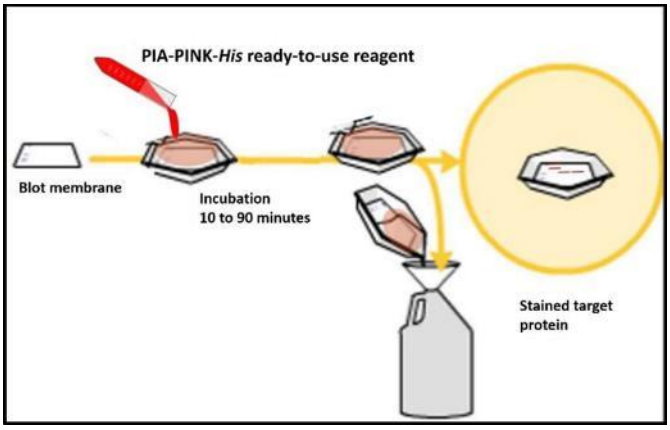
PIA-PINK-*His* ready-to-use reagent is equally suitable for staining nitrocellulose and PVDF.

Implementation

Quick Guide

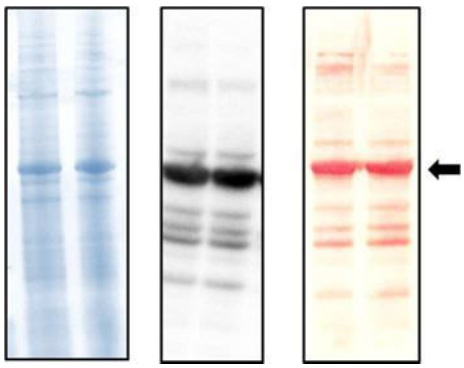
Dried membrane (blot) carrying poly-His-tagged protein

- rinse thoroughly with distilled water
- place into a clean incubation tray
- cover with PIA-PINK-*His* reagent
- incubate in reagent for 10 – 90 minutes
- dry between filter paper
- continue incubation if necessary
- documentation with commercially available camera



Workflow of poly-histidine-labelled protein detection with PIA-PINK-His ready-to-use reagent

Comparison of conventional detection methods and PIA-PINK-His Pico 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: PIA-PINK-His pico (20 minutes)



Detailed Instructions

The PIA-PINK-*His* ready-to-use reagent contains the conjugate of anti-His-tag antibodies and gold nanoparticles in a ready-to-use solution. As the reagent contains no bactericides, the **PIA-PINK-*His* ready-to-use reagent** should be used on the day of opening.

Additional material required: Incubation tray, filter paper, laboratory shaker and camera.

1. Transfer the proteins onto a suitable membrane (western blot, dot blot). Nitrocellulose (NC) and Polyvinylidenfluorid (PVDF) are equally suitable membranes for staining with PIA-PINK products.
2. Let the membrane dry on filter paper to fix the protein. Blowing air over the membrane will speed up the drying process (e.g. using a non-heating blow dryer).
3. Rinse the membrane thoroughly with distilled water.

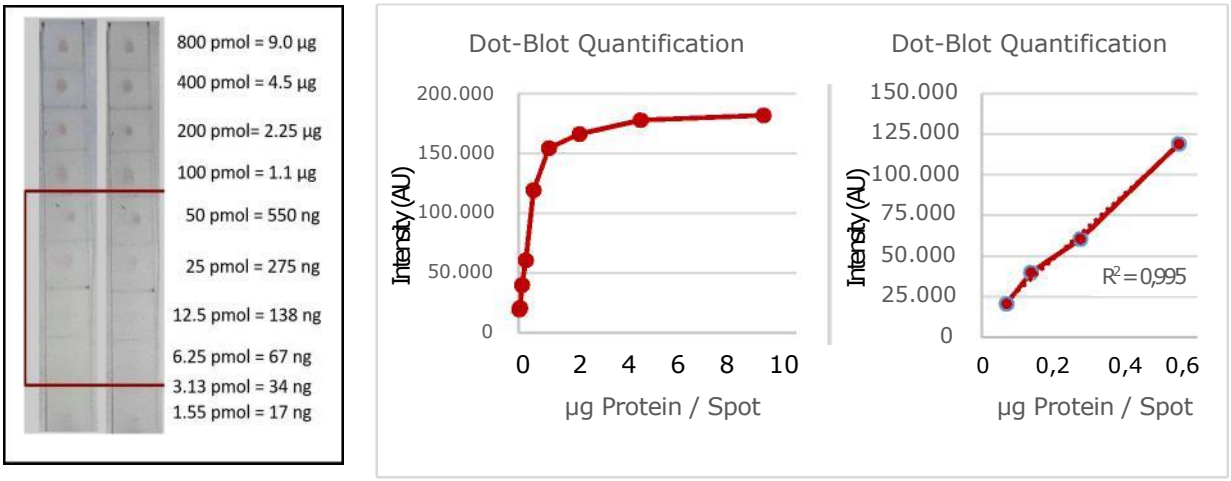
This step is essential for the removal of denaturing agents before the addition of PIA-PINK-*His* ready-to-use reagent!

4. Carefully invert the **PIA-PINK-*His* ready-to-use reagent** to resuspend settled conjugate. The solution is now pale pink.
5. Place the membrane in a clean incubation tray and cover with the **PIA-PINK-*His* ready-to-use reagent**. **It is not necessary to block unspecific binding sites.** (Figure 2)
   To cover a 10 cm x 10 cm blot, a 10 mL aliquot of the reagent is used.
6. Incubate shaking 10 - 30 minutes.
7. Dry the membrane on an absorbent surface before visual analysis and documentation. If desired, continue the incubation by placing the dried membrane back into the reagent.
8. Normally, the staining is complete within 90 Minutes. Further continuing the staining might enhance the staining intensity.
9. For documentation, any commercial photo camera is sufficient.
10. For quantification, any suitable software is sufficient.

Quantification

After 90 minutes incubation with **PIA-PINK-His ready-to-use reagent**, the staining is usually complete. The intensity of the signal directly correlates with the number of binding sites of the antibody. Quantification should be performed using an accompanying standard curve and color matching.

Any suitable software can be used for quantification.



Quantification of poly-histidine labelled protein using PIA-PINK pico 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 PIA-PINK-His pico 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. 1 mm².

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