DATA SHEET

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Particle Immuno Assay PIA-PINK

2nd IgG antibody nanoparticles for Immunoassay

Cat. N°.	Amount
☐ PIA-103-Rabbit	Rabbit 5x Block and Staining Solution (for 5x Blots)
☐ PIA-104-Mouse	Mouse5x Block and Staining Solution (for 5x Blots)

Applications: Western blot, Dot-Blot, ELISA Plate Assay and Electron Microscopy.

Contents of the kit

Reagent 1: 50 mL Blocking solution PIA-PINK-BLOCK

Reagent 2: 50 mL Staining solution PIA-PINK-rabbit (PIA-103) or PIA-PINK-mouse (PIA-104)

Composed of anti-rabbit or mouse IgG antibody (polyclonal, affinity purified, specific against the Fcy fragment) conjugated with gold nanoparticles (Absmax 540 nm)

Sufficient for the immunostaining of five membranes, max. 100 cm2, each. The primary antibody specific for the target protein is not supplied.

For *in vitro* use only!

Shipping:

Ambient temperature (4 °C - 30 °C)

Storage Conditions:

Store at room temperature (15 °C - 25 °C) or in the refrigerator (4 °C - 8 °C); protected from direct sunlight. Do not freeze.

Shelf Life:

6 month, if kept sterile.

Protocol for Western Blot, Dot or Slot Blot

Materials required but not included: primary antibody (IgG), precision pipette, test tubes clean incubation dishes (330 mL flat weighing boats are ideal), filter paper, orbital shaker, opt. digital camera.

1. Hybridisation

Pour 10 mL PIA-PINK staining solution into a 15 mL reagent tube. Add 1 µg primary antibody (1 µL of 1 mg/mL) and immediately mix thoroughly by inverting the tube 10 times (do not vortex). Allow the hybridization to proceed for 15 to 60 minutes before using the reagent.

Important note: In the case the concentration of the primary antibody is not known or the signal is low, you can determine the optimal amount of primary antibody as described in the Supplementary Section below. It is important to use the right ratio 1st and 2nd antibody as excess of primary antibody results in binding competition (less visible signal).

2. Blot-Membrane preparation

Apply the protein to be anaylsed to the membrane using the standards dot blot, slot blot, or western blot. After completing the sample application, place the blot membrane in a clean dish and cover with PIA-PINK-BLOCK (10 mL). Incubate for 5 minutes with vigorous shaking, then pour off blocking solution. Do not reuse blocking solutions.

3. Immunostaining

Cover the blocked membrane with the hybrid solution from step 1 and incubate for one hour while shaking intensively.

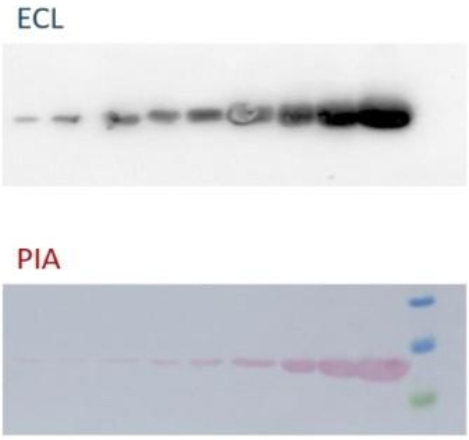
Note: Usually the signal is sufficient after 1-hour incubation. If longer incubation is desired, make sure to avoid evaporation and ensure the blot is constantly covered by the hybrid solution.

4. Detection

Binding of the signaling moiety (hybrid) to the target leads to accumulation of gold nanoparticles on the target protein. The increase in staining intensity can be observed during incubation. When staining is complete, remove the blot from the incubation dish, rinse with water if necessary, and dry on filter paper. The coloration intensifies as the membrane dries.

5. Quantification

The intensity of the staining correlates with the amount of target protein. PIA-PINK results can be quantified by analyzing pixel density on digital images of the blots. Suitable programs such as ImageJ or Image Studio Light can be used for quantification. Some gel documentation systems provide quantification of colorimetric detection. Standard protein dilution must be determined from the same blot. Make sure that the analysis signal is in the linear range. PIA offers you a significantly larger linear range than ECL immunoassays. (Figure 1)



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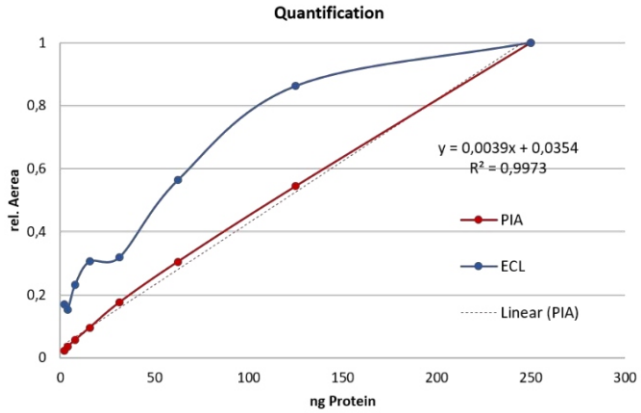

Figure 1: Comparison of ECL immunoassay and PIA by Western Blot and its quantification standard curve of a two-fold dilution series analysis ranging from 250 ng to 2 ng His-tagged TEV protease.

Supplementary Section

Evaluation of the optimal amount of primary antibody for PIA-PINK

1. Dilution of the primary antibody

Dilute 2 µL primary antibody solution with 18 µL 1 x PBS. Fill four 1.5 mL reagent tubes with 150 µL PIA-PINK-BLOCK solution, each. Add the following volumes of the primary antibody dilution and mix by snipping against the tube wall.

a) 0 µL (negative control)
b) 0,5 µL
c) 2,5 µL
d) 12,5 µL

This dilution test is suitable for primary antibody concentrations from 0,1 mg / mL to 5 mg / mL.

2. Sample preparation

Select a representative test protein sample and ideally a negative control sample.
Cut an approx. 2 cm x 5 cm strip from a suitable blot membrane. Mark four fields and two spot positions (+ and -) on the strip, using a soft pencil. (Figure 2)
Apply 2 µL from each protein sample on the respective position and let the drops dry dry and cut the membrane into four pieces as shown in figure 2.

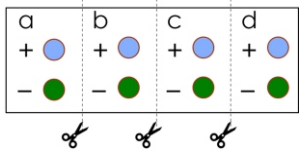

Figure 2: Test strip for primary antibody validation. 2 µL test and control protein is spotted onto a suitable membrane, e.g. nitrocellulose or PVDF. The latter hydrated according to the manufacturer's instruction. The zones, spots and cutting lines are marked with a soft pencil.

Select four small and clean trays and label them with a) to d). Flat 3 ml weighing boats, for example, are well suited. Place each of the four membrane strips in a clean incubation dish and cover them with 1 ml PIA-PINK-BLOCK, each. Incubate the strips at room temperature for 5 minutes on a shaker. Discard the blocking solution.

3. Immunoassay

Add the respective antibody solution a) to d) from step 1) to the respective incubation tray. Start with a) to avoid contamination of the control sample. Also avoid accidental contamination from splashes, pipette tips, gloves or tweezers. Incubate the membranes in the incubation trays for one hour at room temperature while shaking intensively. Remove the membranes from the solution (starting with a) and dry on filter paper.

4. Evaluation:

Control (a) should show no staining. If staining occurs, please repeat the test first, making sure that no primary antibody is inadvertently introduced into well a). If you are sure that the background staining is genuine, please contact us. The test batches b) to d) should show different color intensities on the positive sample spots. The dilution with the most intense staining on the + zone and no staining on the - zone is the one best suitable for PIA. This dilution should be used for all subsequent PIA staining with this primary antibody.

Dilution	Primary Antibody		PIA-PINK-MOUSE
a.	2,0 µL PBS		10 mL
b.	2,0 µL 1/10 diluted		10 mL
c.	1,0 µL original stock		10 mL
d.	10 µL original stock		10 mL

Table 1: Transfer of the evaluation results to the volume required for Western blots