Produkt Information

PiNa-Tec
Bio-Nanotechnologie

PIA-PINK-COMFORT KIT

Ready-to-use immunostaining reagent kit based on nanogold labelled secondary antibodies.

Produktname	Produktnummer	Symbol	Inhalt	Volumen
PIA-PINK-COMFORT KIT **Rabbit**	# 103-21		PIA-PINK-COMFORT Rabbit Staining Solution	1 x 50 mL
			PIA-PINK-Block Blocking Solution	1 x 50 mL
PIA-PINK-COMFORT KIT **Mouse**	# 104-21		PIA-PINK- COMFORT Mouse Staining Solution	1 x 50 mL
			PIA-PINK-Block Blocking Solution	1 x 50 mL
PIA-PINK-COMFORT KIT **Human**	# 105-21		PIA-PINK- COMFORT Human Staining Solution	1 x 50 mL
			PIA-PINK-Block Blocking Solution	1 x 50 mL

Content of the Kits

Each PIA-PINK-SPEED KIT contains 50 mL Staining Solution and 50 mL Blocking Solution. The Staining Solution contains nanogold labelled goat polyclonal antibodies. Currently, three anti-IgG-Fcγ specificities are available from stock: anti-rabbit, anti-mouse and anti-human.

The solutions in one kit are sufficient to cover 500 cm^2 of membrane, which is sufficient to analyze 520 samples derived from five 96-well plates, along with eight standard or control samples.

PIA-PINK Immunoassay

In contrast to the multi-step procedure of classical immunoassays, the particle immunoassay (PIA) is performed in a time-saving manner without washing steps. After a short preparation of the membrane for staining by blocking the free binding sites, the antibody incubation and staining is performed in only one step. The primary antibody incubation is performed with the hybrid of the primary antibody and the PIA-PINK solution. PIA-PINK saves time, effort and valuable primary antibody and provides real-time staining with a visible and quantitative signal in a robust, flexible and reliable manner. (Figure 1)

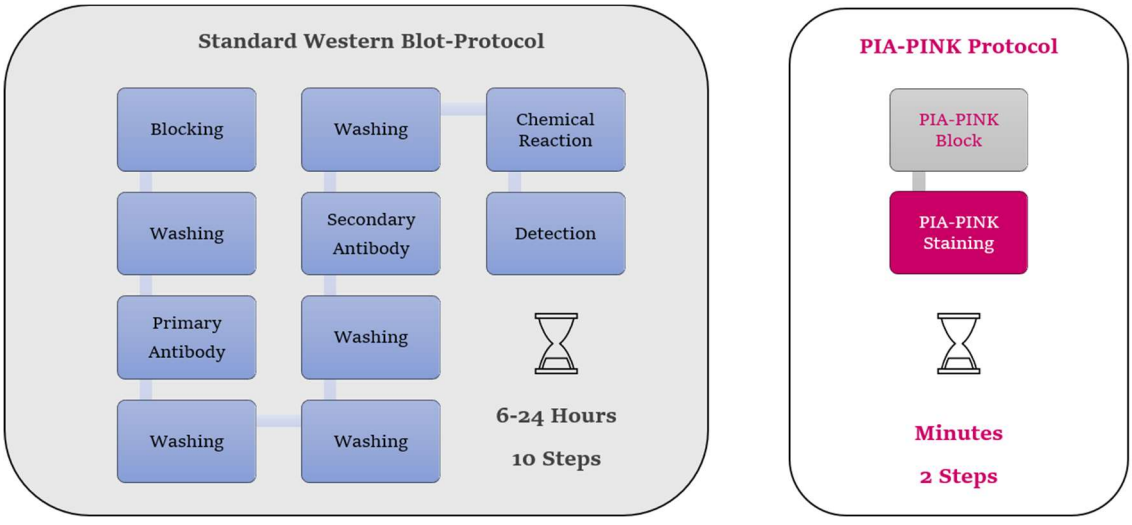


Figure 1: Left: Classical immunoassay with 10 steps and 6 to 24 hours to result, depending on the protocol. Right: Procedure of the 2-step particle-based immunoassay with PIA-PINK. The result is visible within minutes.

APPLICATIONS OF PIA-PINK-COMFORT

PIA-PINK-COMFORT is optimized for use in Western Blot Immunoassays. The visible accumulation of nanogold particles occurs in real time, so that a sample with a high concentration can be identified very quickly. For example, bands containing 30 pmol epitope/µL can be detected within 15 minutes, such as a band created from 3 µL of a 30 kDa protein of 1 µg/µL with one antibody binding site, each. Less concentrated bands follow.
1 pmol antigen per µL sample is usually detected within 60 minutes (e.g. 30 ng/µL for a 30 kDa protein).
The detection limit for PIA-PINK reagents is approximately 30 fmol/µL antigen (1 ng/µL for a 30 kDa protein). PIA-PINK-COMFORT can also be used in dot blot or slot blot immunoassays. In addition to visual assays, PIA-PINK products are suitable for other immunological detection, e.g. by high electron density or light scattering. PIA-PINK can be used for the detection of antibodies or antigens.

Antigen Immunoassay

PIA-PINK-COMFORT allows antigen detection in a single antibody incubation step. The primary antibody is mixed with PIA-PINK-COMFORT prior to the immunoassay. Spontaneous and directed binding generates a visible, antigen-specific molecular probe. Figure 2 and Figure 3 schematically show the hybridization of PIA-PINK Mouse with primary IgG antibodies from murine cultures. The primary antibodies are used very sparingly. Already 0.5 pmol (0.07 µg) primary antibodies per milliliter PIA-PINK-COMFORT Staining Solution form saturated hybrids with 80 primary antibodies per nanogold particle. Due to the high dilution of 1/15,000 of a primary antibody of 1 mg/mL, excellent specificity is achieved. Due to the spatial arrangement of the primary antibodies on the nanogold particles, rapid detection is achieved despite the high dilution.

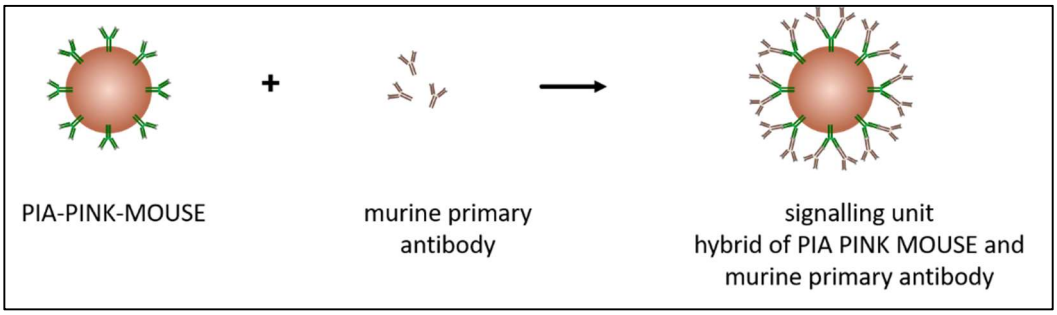


Figure 2: Hybridization of primary antibodies from mouse cultures with PIA-PINK Mouse Staining Solution. The red nanogold conjugated anti-mouse IgG secondary antibody binds to the Fcγ fragment of the primary antibody. The specific region of the primary antibody required for antigen binding remains freely accessible.

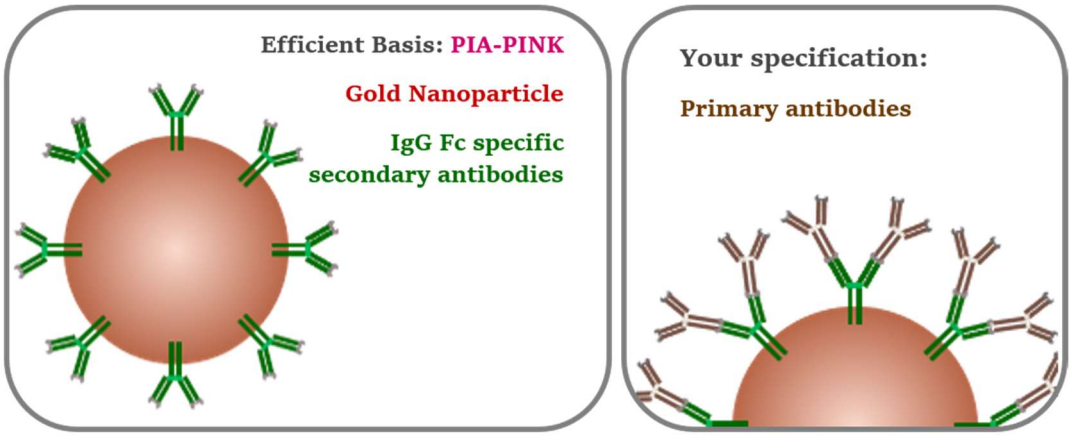


Figure 3: Close up at the PIA-PINK primary antibodies hybrid.

Dot Blot Analysis of Antigen Proteins

The hybrids of PIA-PINK and primary antibodies can be used for protein analysis in immunoblots. Figure 4 shows an example of the optical evaluation of a qualitative protein analysis performed as shown in Figure 5. Figure 6 shows the quantitative analysis of this PIA-PINK Western blot compared to enhanced chemiluminescence (ECL) assay.

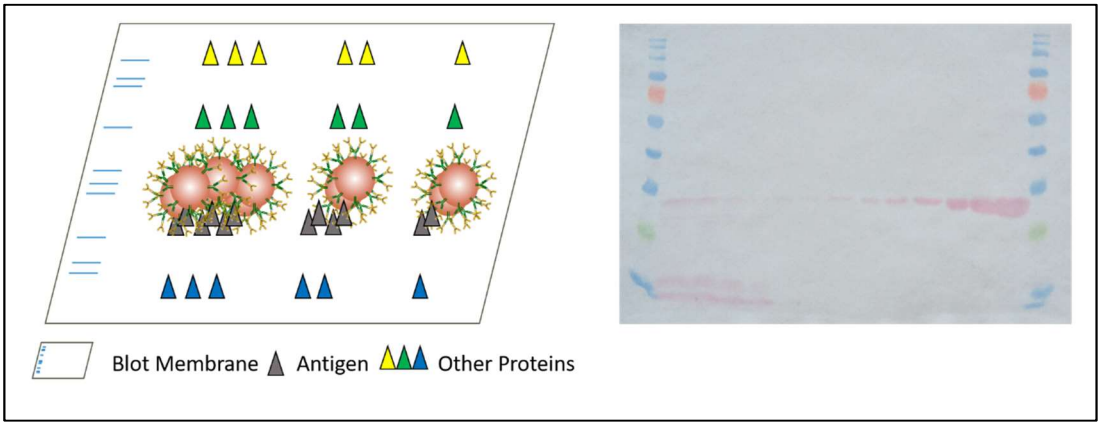


Figure 4: Antigen binding of hybrids of primary antibodies and PIA-PINK-COMFORT in Western blot. Western blot on NC. Expression experiments with poly-histidine-labeled Tev protease (lanes 2-5) and concentration series of purified Tev protease for quantification (lanes 6-14), molecular weight markers in lanes 1 and 15. Free binding sites were saturated by incubation with PIA-PINK-BLOCK for 5 minutes. Immunostaining was performed with the hybrid of mouse anti-His tag IgG and PIA-PINK-COMFORT mouse. Immunostaining was performed on an orbital shaker at 100 rpm for 1 hour. Camera: Canon 400D.

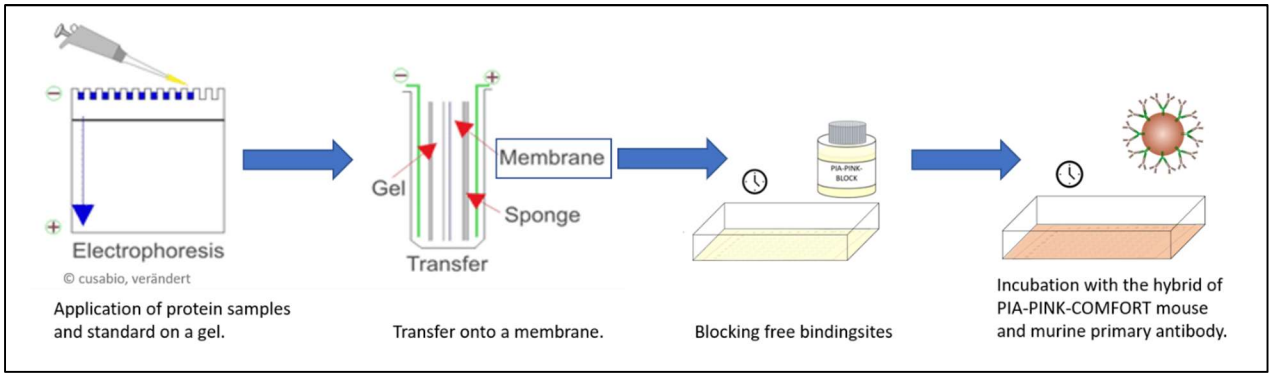


Figure 5: Western blot analysis of an expression screen with four conditions. Schematic of the assay procedure with electrophoretic separation of samples, transfer to blotting membrane, blocking and immunostaining.

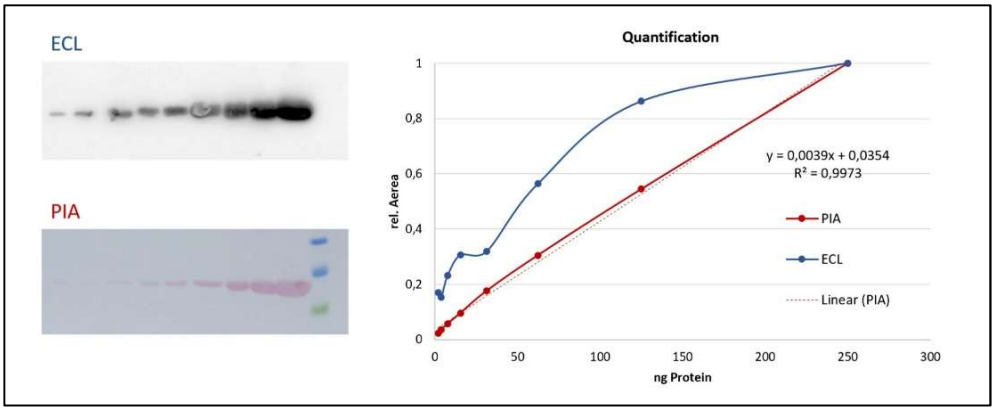


Figure 6: Quantification of a Western Blot Dilution Series. Comparison between ECL and PIA-PINK Immonoassay 250 ng to 1 ng H6-Tev protein was separated by 10% SDS-PAGE and blotted onto NC. One NC was treated with the ECL protocol and one with the PIA-PINK-COMFORT protocol. ECL (top left): blocking with 1% BSA in 1 x TBS-T for 1 h; washing with 1 x TBS-T for 15 min; incubation with mouse anti-poly-His-Tag antibody 1: 1,000) in 10 mL 1 % BSA in 1x PBS for 16 h in the cold room; wash 3 times 10 min each; incubate with 2. antibody goat anti-mouse His-Tag antibody (1:1,000) in 10 mL 1 % BSA in 1x PBS for 16 h in the cold room; incubate with 3. antibody goat anti-mouse His-Tag antibody (1:1,000) in 10 mL 1 % BSA in 1x PBS for 16 h in the cold room; incubate with 4. Antibody Goat anti-mouse HRP antibody (1:10,000) in 10 mL 1 % BSA in 1x PBS 1 h; 3 x washing á 10 min; chemical reaction: 6 mL Clarity Western ECL Substrate; Detection and documentation: ChemiDoc MP Imaging System 5 min. Total assay time ECL: 19 h. PIA (bottom left): as described inFigure 4. Total assay time PIA: 70 min. Quantification (right) PIA-PINK shows good linearity.

Western Blot Analysis of Antibody Proteins

When analyzing antibodies, the PIA-PINK Staining Solution can be used without hybridization. Figure 7 shows an example of immunodetection of antibody samples analyzed by Western Blot. Binding occurs on the native, denatured and reduced γ fragment of the Fc portion of the heavy chain for all IgG subtypes. Reducing or non-reducing, native or SDS-PAGE Western blots can therefore be used to make specific statements about the quaternary structure of the antibodies under analysis. This is particularly attractive for quality assurance of antibody production samples.

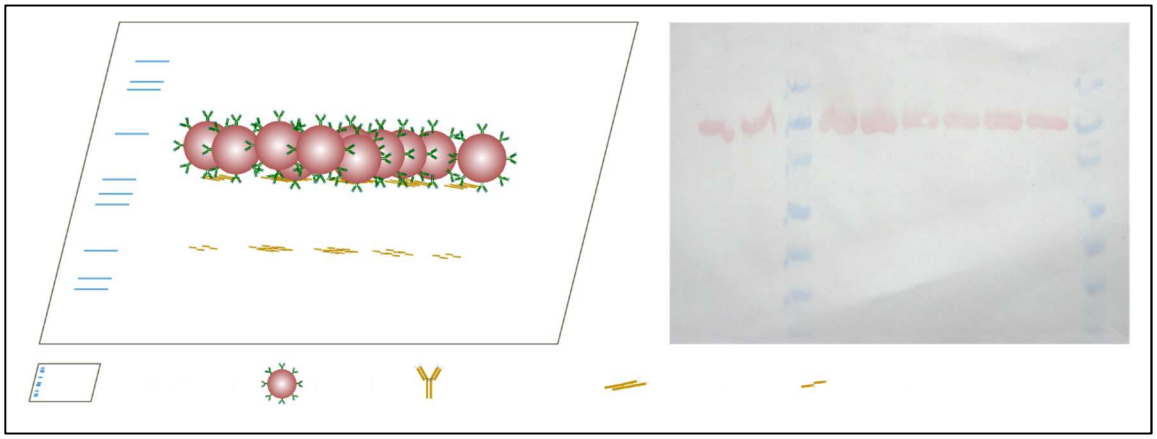


Figure 7: Left: Illustration of the direct binding of PIA-PINK-COMFORT to the heavy chain of the corresponding target antibody at the level of the 50 kDa band in the Western blot after reducing SDS-PAGE. Right: Western blot on cellulose nitrate (NC) after 12% SDS-PAGE under reducing conditions. Application of different purification fractions of humanized antibody (Herceptin). Free binding sites were saturated by incubation with PIA-PINK-BLOCK for 5 minutes. Staining with PIA-PINK-COMFORT human, 1 h RT; 100 rpm on rotary shaker.

The binding activity of antibodies against different antigens can be investigated with PIA-PINK-SPEED in a simple membrane spot test. (see PIA-PINK-SPEED instructions) and can be precisely localized in a Western blot with PIA-PINK-COMFORT.

PIA-PINK-COMFORT Quick Guide

Immunostaining of ANTIBODIES in Western Blot Assays

Preparatory Work

- Fill the required volumes of PIA-PINK-COMFORT KIT solutions into clean tubes. Ensure that the remaining KIT solutions remain sterile. Equilibrate the required solutions to room temperature.

Immunostaining with PIA-PINK-COMFORT after Protein Blotting on the Membrane

- Place the blot membrane in a clean dish and incubate covered with PIA-PINK-BLOCK Blocking Solution for at least 5 minutes with shaking. Discard the solution.
- Cover the blot membrane with PIA-PINK-COMFORT Staining Solution and incubate for 1 hour with shaking. During the incubation, the enrichment of the immunostaining can be observed.
- Remove and dry the membrane.
- Evaluate and photograph the staining, continue the incubation if necessary.

PIA-PINK-COMFORT Quick Guide

Immunostaining of ANTIGENES in Western Blot Assays

Preparatory Work

- Fill the required volumes of PIA-PINK-COMFORT KIT solutions into clean tubes. Ensure that the remaining KIT solutions remain sterile. Equilibrate the required solutions to room temperature.
- Mix the primary antibody with PIA-PINK-COMFORT Staining Solution and allow it to hybridize for one hour. Ideal hybrids require 0.07 µg of primary antibody per mL of PIA-PINK-COMFORT Staining Solution.

Immunostaining with PIA-PINK-COMFORT-Hybrid after Protein Blotting on the Membrane

- Place the blot membrane in a clean dish and incubate covered with PIA-PINK-BLOCK Blocking Solution for at least 5 minutes with shaking. Discard the solution.
- Cover the blot membrane with PIA-PINK-COMFORT primary antibody hybrid solution and incubate for 1 hour with shaking. During the incubation, the enrichment of the immunostaining can be observed.
- Remove and dry the membrane.
- Evaluate and photograph the staining, continue the incubation if necessary.

Detailed Instructions for PIA-PINK-COMFORT Immunoassays

These instructions explain how to use PIA-PINK-COMFORT in a Western Blot immunoassay for an NC membrane Midi-Blot (11 x 9 cm), which requires 10 mL to cover the membrane. If you use a larger or smaller membrane, adjust the volumes accordingly, ensuring that the membrane is continuously covered with PIA-PINK solution.

Additional materials required: (optional) IgG type primary antibodies (affinity purified), a precision pipette, two 15 mL reagent vials, a clean incubation dish, filter paper, a shaker, and a digital camera.

Preparatory Work

PIA-PINK Filling for Sterility Preservation

Before use, fill 10 mL of each of the two PIA-PINK-COMFORT KIT solutions into a clean 15 mL tube and allow them to reach room temperature. Ensure that the remaining KIT solutions remain sterile. To ensure the sterility of the remaining solution, it can be sterile filtered again using a 0.45 µm PES filter.

Hybridization of Primary Antibodies with PIA-PINK-COMFORT Staining Solution

To detect antigens, first hybridize the corresponding PIA-PINK Staining Solution with primary antibodies. If you are detecting antibodies, skip this step.

Fill a 15 mL reaction vial with 10 mL of PIA-PINK-COMFORT. Then, add 0.7 µL of a 1 mg/mL primary antibody solution and mix well by inverting the vial several times. If the primary antibody solution is higher concentrated, dilute it to 1 mg/mL with 1x PBS or PIA-PINK-BLOCK Blocking Solution. Finally, incubate the solution for 1 hour with shaking to obtain a homogeneous hybrid solution. (Figure 8)

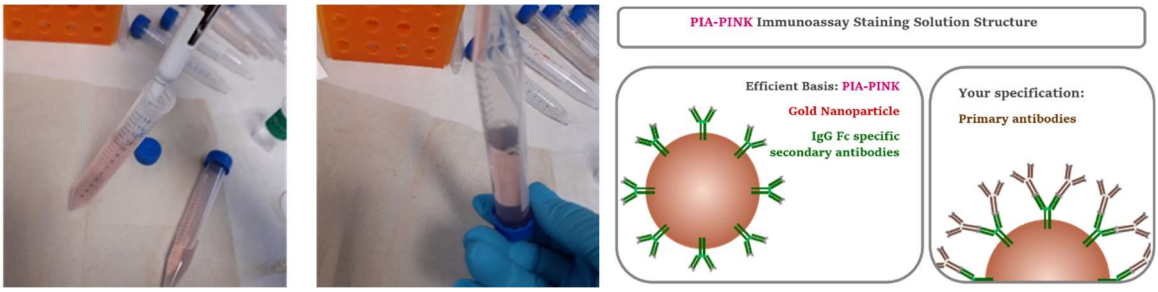


Figure 8: Preparation of the hybrid solution by adding the primary antibody to PIA-PINK-COMFORT.

PIA-PINK is designed to detect IgG-type antibodies exclusively, excluding other globulins like IgA, IgM, and IgE that are not hybridized.

If non-affinity-purified IgG primary antibody mixtures, such as sera or ascites fluid, are used, there may be competition for binding sites on the nanogold labelled secondary antibody. If it is not feasible to use affinity-purified primary antibodies and the desired staining is not observed, try reducing the amount of primary antibody used as a first measure. Excessive primary antibodies can interfere with detection, as the antigen becomes blocked by the excess antibody.

Please use no more than 0.07 µg of primary antibodies per mL of PIA-PINK-SPEED. If in doubt, use less primary antibodies as access competes with the antigen staining. If the antibody concentration is unknown, test 1/20 of the dilution recommended by the manufacturer for dot blots. Ideally use affinity purified antibodies.

Western Blot

After gel electrophoresis, transfer the proteins to a blotting membrane, such as a cellulose nitrate (NC) or PVDF membrane, according to your standard protocol. After transfer, blot gel residues from both sides of the blotting membrane under running water.

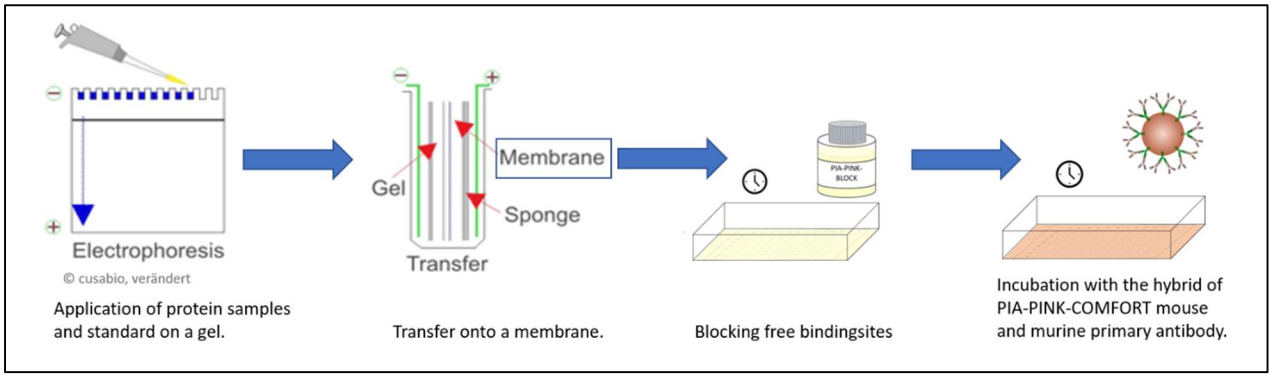


Figure 9: Western blot analysis of an expression screen with four conditions. Schematic of the assay procedure with electrophoretic separation of samples, transfer to blotting membrane, blocking and immunostaining.

1. Probenauftrag

For Western blot analysis, samples are separated by gel electrophoresis and transferred to blotting membranes. Cellulose nitrate (NC) and PVDF membranes are equally suitable for the PIA-PINK PINK Immunoassay. After transfer, remove residual gel fragments and SDS from both sides of the blotting membrane by gently rinsing with tap water.

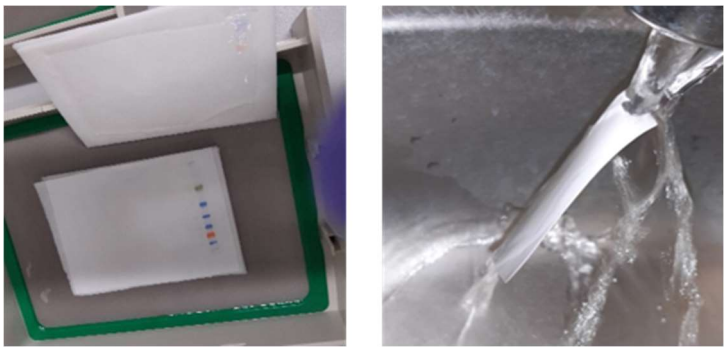


Figure 10: After transfer, rinse the blotting membrane with water to remove SDS and residual gel fragments.

2. Blocking of Free Protein Binding Sites on the Membrane

Place the Western blot membrane in a clean dish and cover with 10 mL PIA-PINK-BLOCK. Saturate free protein binding sites by incubating for at least 5 minutes with vigorous shaking. Then discard the blocking solution.

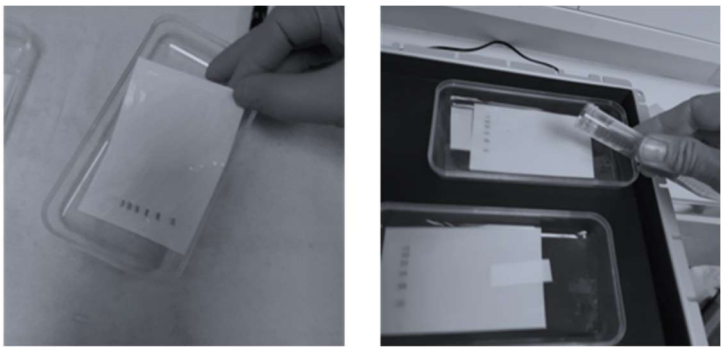


Figure 11: Incubation of the blot membrane with PIA-PINK-BLOCK blocking solution.

3. Immunostaining and Detection

Cover the nitrocellulose (NC) membrane with 10 mL of PIA-PINK-COMFORT primary antibody hybrid and incubate for 1 hour with constant shaking at 50 to 75 revolutions per minute (rpm) on a rotary shaker. The hybrid binds to the antigen, causing red nanogold particles to accumulate on the band. Slower shaking will prolong the incubation time. The degree of enrichment depends on the epitope concentration and the incubation time. For instance, immunostaining of 30 pmol of antigen (equivalent to 1 µg of 30 kDa) becomes visible within 15 minutes, while less concentrated spots take longer. The average incubation time required to detect 1 pmol of antigen (30 ng for a 30 kDa protein) per band using PIA-PINK-COMFORT is 1 hour. The detection limit for PIA-PINK is approximately 30 fmol of antigen (equivalent to 1 ng of a 30 kDa protein) per band. Color intensity can be observed and photographed during incubation. Immunostaining can be stopped or interrupted at any time and resumed if necessary. Generally, 1 hour of incubation with PIA-PINK-COMFORT is sufficient to produce a signal. If a longer incubation is desired, cover the tray to prevent evaporation and ensure that the blot remains constantly covered by the hybrid solution to avoid drying effects. To document the process, remove the blot from the incubation dish and dry it on filter paper. The membrane will initially appear pink, but will discolor during drying and the band coloration will become more intense. The blot can be photographed with a digital camera or recorded in the gel documentation system using the 'Ponceau red staining' setting. (Figure 12)

Figure 12: Immunostaining with the hybrid solution of PIA-PINK-COMFORT Mouse and murine primary antibody.

Quantification

The pixel density on digital images of the blots can be analyzed to quantify the PIA-PINK results. Programs such as ImageJ or Image Studio Light are suitable for this purpose. Some gel documentation systems also offer software for quantifying colorimetric detection.

It is important to determine the standard protein dilution and analyte from the same blot. PIA-PINK has a linear range of detection that spans 1 to 2 orders of magnitude. It is recommended to capture multiple images of the same blot during the staining process. This allows for flexible coverage of the detection range and reliable determination of analytes with unknown concentrations within the linear range.

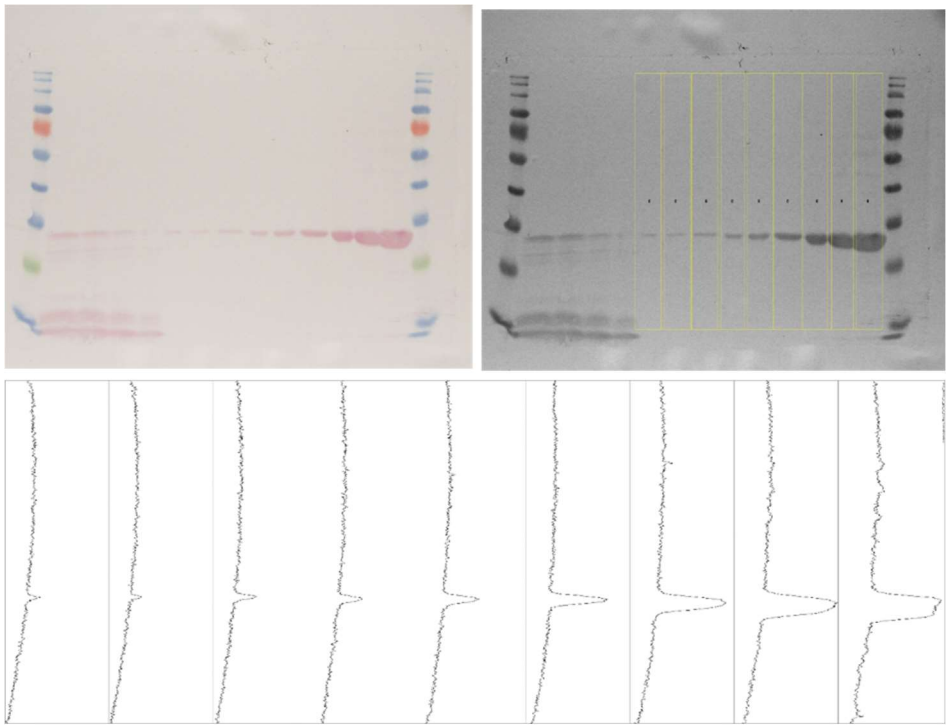
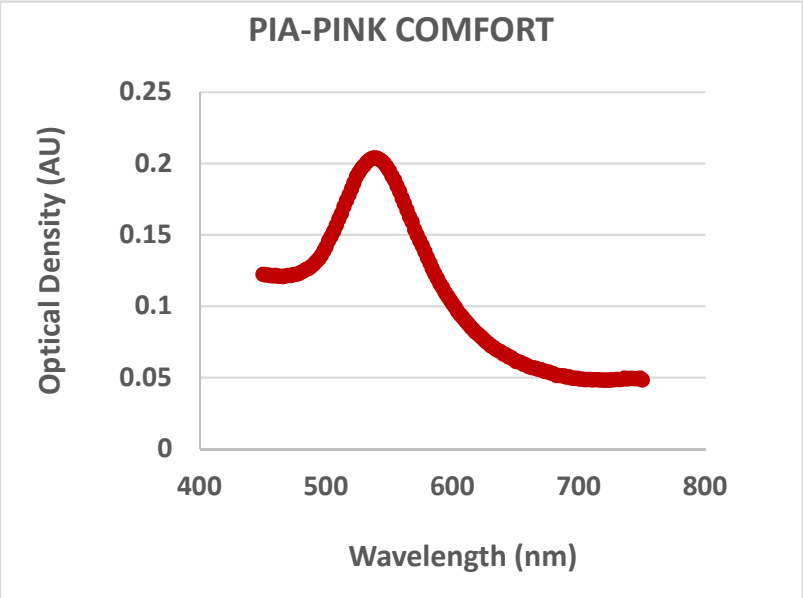


Figure 13: Example of quantification using ImageJ. Top left: Jpg file of the photographed Western blot from Figure 4. Upper right: Image in 8-bit grayscale format with selection of columns (lanes). Bottom: Line plots of the pixel intensities of the respective lanes. After defining the baseline, the peak area is determined, assigned to the known plotted concentrations and displayed graphically. A trend line is created and described mathematically, here using Microsoft Excel. (See Figure 6) The equation can be used to quantify the peak areas of the samples.

Product Characteristics

Article	**PIA-PINK-COMFORT KIT**
Description	Ready-to-use solutions for immunological detection. The kit consists of a blocking solution and a staining solution containing goat anti-IgG-Fc secondary antibodies conjugated with Nanogold. Alternative to enzyme- or dye-labeled antibodies.
Antibody	Goat polyclonal anti-IgG-Fc, affinity purified # 103-21 Goat-Anti Rabbit IgG (Fc) # 104-21 Goat-Anti Mouse IgG (Fc) # 105-21 Goat-Anti Human IgG (Fc)
Specificity	Reacts with IgG from the corresponding species. Binds to the Fc part of the heavy chain, but not to Fc-free derivatives. Binding to other serum proteins was not detected. Cross-reactivity with sera of the other species below 1 %.
Conjugation	60 nm nanogold particles; max. absorption at 540 nm. OD (540) 0,2. 
Other Components	Wasser, BSA, PBS, Tween 20, Citrate @ pH 7.4
Recommended Applications	Western Blot
Further Applications	Dot Blot, Slot Blot, Vertical-Flow-Assay, Electron microscopy, Darkfield microscopy

Product Form	This package includes two 50 mL bottles of ready-to-use solutions: PIA-PINK-COMFORT staining solution and PIA-PINK-BLOCK blocking solution. The provided quantity is enough for immunostaining 500 cm2 of membrane. It is recommended to use at least 0.1 ml per cm2 of incubation area to ensure uniform and complete coverage of the membrane and an adequate amount of conjugate. The user should add the primary antibody specific to the antigen according to the provided instructions. For best results, use 0.07 µg of affinity-purified IgG primary antibody per mL of PIA-PINK-COMFORT Staining Solution. Begin hybridization at least 1 hour before use.
Transport	Ambient temperature (4 °C - 30 °C)
Storage	The assay required volume can be stored at room temperature for up to one week. For longer storage, refrigerate between 4°C and 8°C. Do not freeze! Protect the solution from direct sunlight and xenon light.
Shelf Life	1 year from date of receipt. The expiry date may be extended if the test results are acceptable for the intended use.

For research and development only, not approved for medical applications.

For technical support, please contact info@pina-tec.de.

Valid from 22th of February 2024

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Bio-Nanotechnologie

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