Product Information

PiNa-Tec
Bio-Nanotechnologie

PIA-PINK-SPEED KIT

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

Article	Catalogue No.	Icon	Content	Quantity
PIA-PINK-SPEED KIT **Rabbit**	# 103-31		PIA-PINK-SPEED Rabbit Staining Solution	1 x 50 mL
			PIA-PINK-Block Blocking Solution	1 x 50 mL
PIA-PINK-SPEED KIT **Mouse**	# 104-31		PIA-PINK- SPEED Mouse Staining Solution	1 x 50 mL
			PIA-PINK-Block Blocking Solution	1 x 50 mL
PIA-PINK-SPEED KIT **Human**	# 105-31		PIA-PINK- SPEED 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)

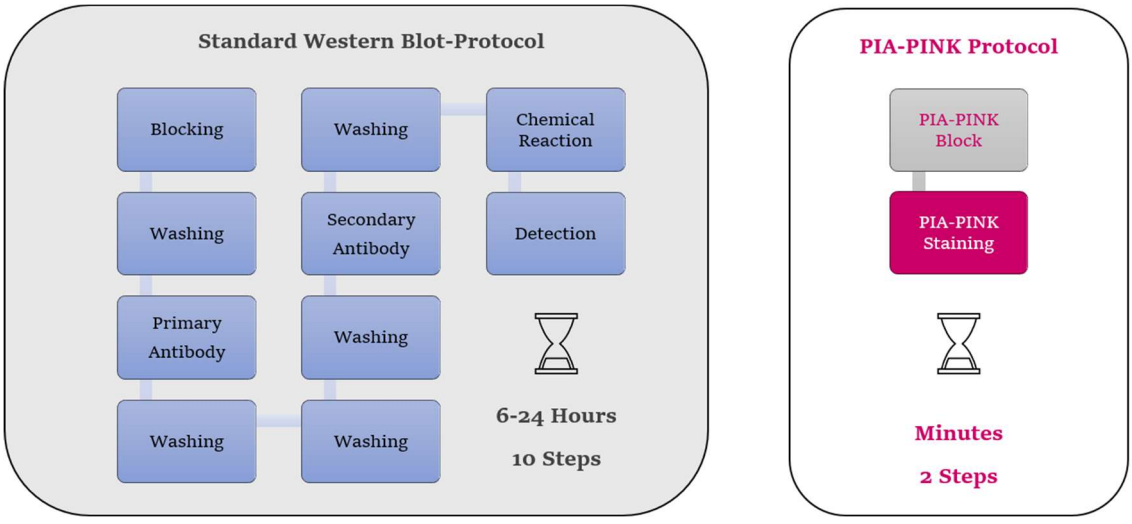

Figure 1: Left: Classical immunoassay with 10 steps and 6 to 24 hours to result, depending on the protocol. Right: Procedure of the 2-step particle-based immunoassay with PIA-PINK. The result is visible within minutes.

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APPLICATIONS OF PIA-PINK-SPEED

PIA-PINK-SPEED is optimized for use in dot blot or slot 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, spots containing 30 pmol epitope/µL can be detected within 5 minutes, such as a 3 µL spot containing 1 µg/µL of a 30 kDa protein with one antibody binding site. Less concentrated spots follow. 1 pmol antigen per µL sample is detected within 15 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-SPEED can also be used for Western blotting. 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-SPEED allows antigen detection in a single antibody incubation step. The primary antibody is mixed with PIA-PINK-SPEED 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 1.5 pmol (0.2 µg) primary antibodies per milliliter PIA-PINK-SPEED Staining Solution form saturated hybrids with 80 primary antibodies per nanogold particle. Due to the high dilution of 1/5,000 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.

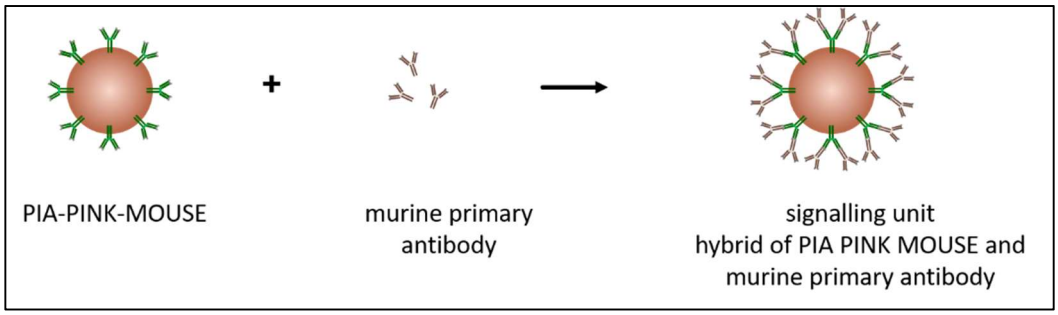


Figure 2: Hybridization of primary antibodies from mouse cultures with PIA-PINK Mouse Staining Solution. The red nanogold conjugated anti-mouse IgG secondary antibody binds to the Fcγ fragment of the primary antibody. The specific region of the primary antibody required for antigen binding remains freely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

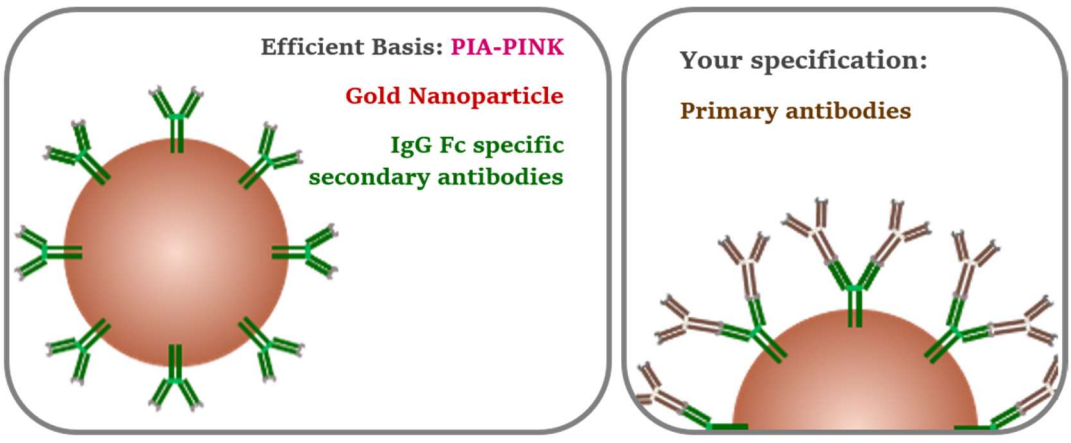


Figure 3: Close up at the PIA-PINK primary antibodies hybrid.

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. Figure 5 and Figure 6 show examples of optical evaluation of quantitative protein analysis of samples in 96-well screening format.

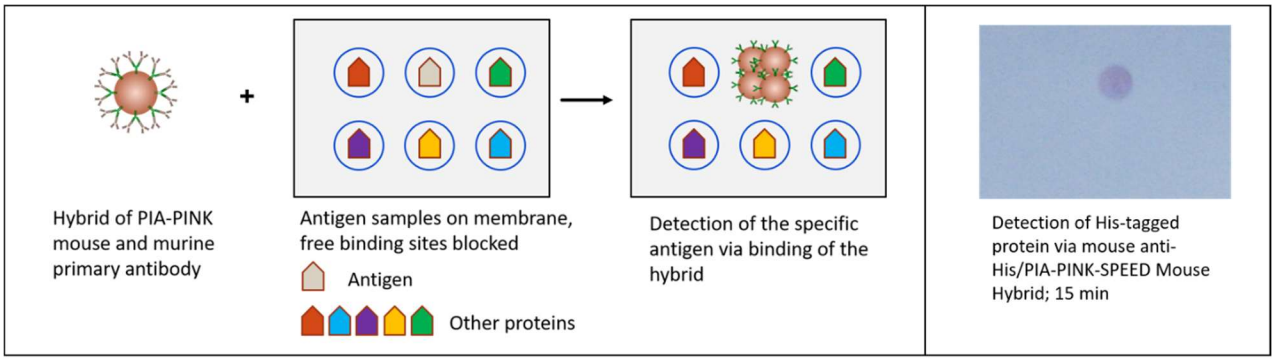


Figure 4: Left: Schematic representation of the qualitative antigen dot blot. The hybrids of primary antibody and PIA-PINK bind to the antigen. Right: Dot blot on cellulose nitrate (NC) with different protein samples from the purification of a His-tagged Tev protease (6 samples of 1.5 µL each in 2 rows). Free binding sites were saturated by incubation with PIA-PINK-BLOCK for 2 minutes. The antigen containing sample in the center of the top row is colored pink by binding of the hybrid. Immunostaining was performed with mouse-anti-His-PIA-PINK-SPEED mouse hybrid for 15 minutes at RT on an orbital shaker at 100 rpm. Photograph: Canon 400D.

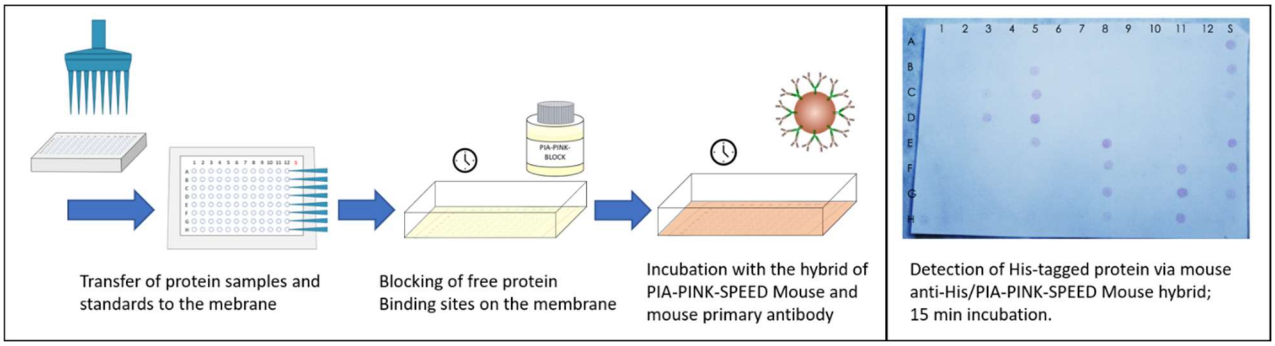


Figure 5: Dot blot analysis of a 96-well expression screen. Left: Schematic of the assay procedure including sample addition, blocking and immunostaining. Right: Photo of a PIA-PINK dot blot assay after 30 minutes of staining with PIA-PINK-SPEED Hybrid. Lanes 1 through 12 correspond to lysate samples from the 96-well plate. Lane "S" contains the standard samples: positions A and E: 150 ng/µL; B and F: 75 ng/µL; C and G: 37.5 ng/µL; D and H: 18.75 ng/µL). PIA-PINK-BLOCK 2 minutes. Immunostaining with mouse anti-His-PIA-PINK-SPEED mouse hybrid for 30 minutes at RT on an orbital shaker at 100 rpm. Photography: Canon 400D.

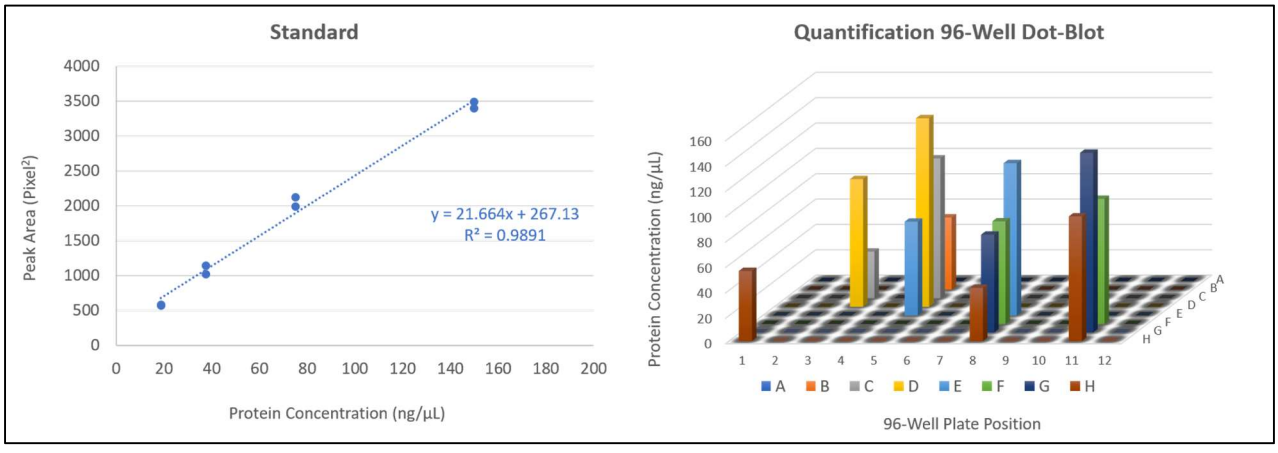


Figure 6: The expression screening in 96-well format from Figure 4 was quantified using dot blot analysis. The peak areas were determined by employing image analysis software (specifically, ImageJ) and assigned to the known plotted concentrations. The results were displayed graphically, and a trend line was created and described mathematically using Microsoft Excel. The equation was then used to quantify the peak areas of the sample spots. The expression rate was highest under the conditions of D5, with approximately 150 ng/µL. The protein levels for G11, E8, and C5 were 140 ng/µL, 120 ng/µL, and 110 ng/µL, respectively. D3, F11, and H11 all have protein levels of 100 ng/µL. F8, G8, E5, B5, H1, and C3 had protein levels ranging from 80 ng/µL to 35 ng/µL. For all other conditions, the target protein was below the detection limit of this blot, which is approximately 20 ng/µL.

Dot 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 Dot Blot. Binding occurs on the native, denatured and reduced γ fragment of the Fc portion of the heavy chain for all IgG subtypes. The easy visual detection is particularly attractive for the analysis of samples from antibody production, immunization or host species identification.

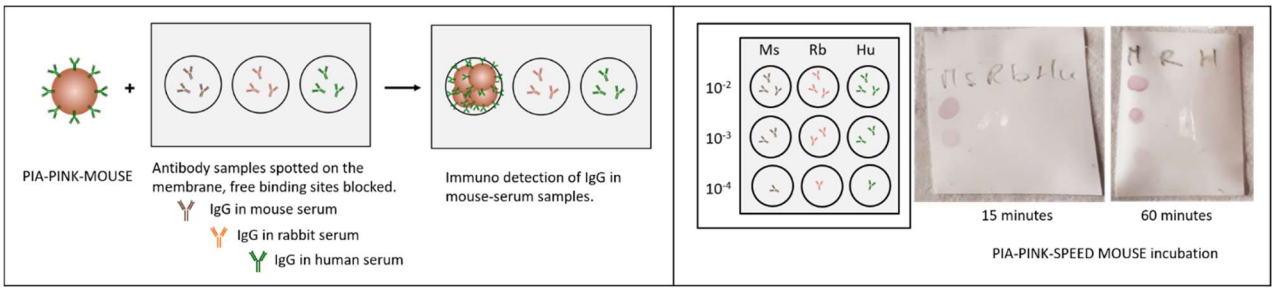


Figure 7: Left: Illustration of the direct binding of PIA-PINK-MOUSE to the corresponding target antibodies in the dot blot. Right: Dot blot on cellulose nitrate (NC). Application of 1.5 µL serum dilution each. Top: 1/100; middle: 1:1,000; bottom 1:10,000. From left to right: mouse, rabbit and human serum. PIA-PINK-BLOCK for 2-minutes. Staining with PIA-PINK-SPEED Mouse on a shaker. Left 15 minutes, right 60 minutes. Photo: Samsung Galaxy S40

Antibody Activity Assay

The binding activity of antibodies against different antigens can be examined with PIA-PINK in a simple membrane spot test. This allows to compare different variants or batches or to identify relevant binding partners, Figure 8 and Figure 9 show examples of the binding activity of different Herceptin (humanized anti-HER2neu antibody) purification variants against the Her2 antigen.

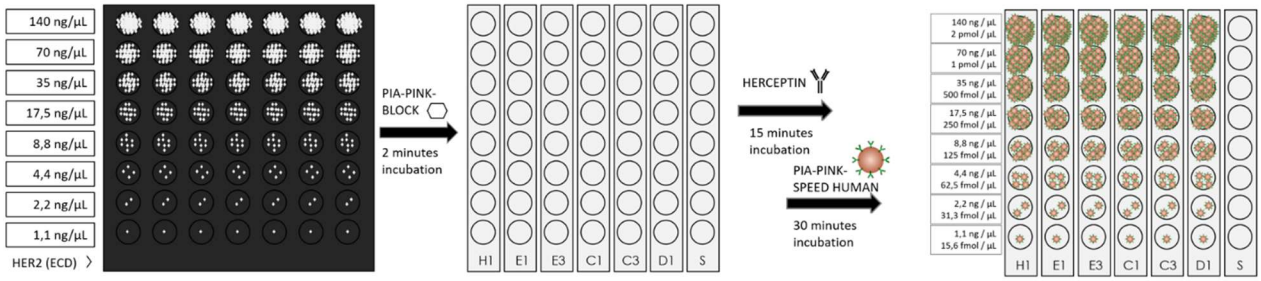


Figure 8: Procedure of an antibody activity assay with PIA-PINK-SPEED using variants of the anti-HER2 antibody as an example. Application of 3 µL antigen (Her2 ECD) on cellulose nitrate (NC). From top to bottom in decreasing concentration (140 ng/µL to 1.1 ng/µL corresponding to 2 pmol/µL to 30 fmol/µL). Free protein binding sites were blocked with PIA-PINK-BLOCK for 2 minutes. The blocking solution was discarded and the NC was cut into strips and labeled. H1: Herceptin Original; E1, E3, C1, C3 and D1: Herceptin purified according to different protocols; S: control for non-specific binding of PIA-PINK to HER2. The strips are incubated with Herceptin 2 µg in 3 mL PIA-PINK BLOCK for 15 minutes. The HERCEPTIN solutions were spiked with 7 mL PIA-PINK-SPEED Human. Immunostaining with PIA-PINK was performed for 30 minutes.

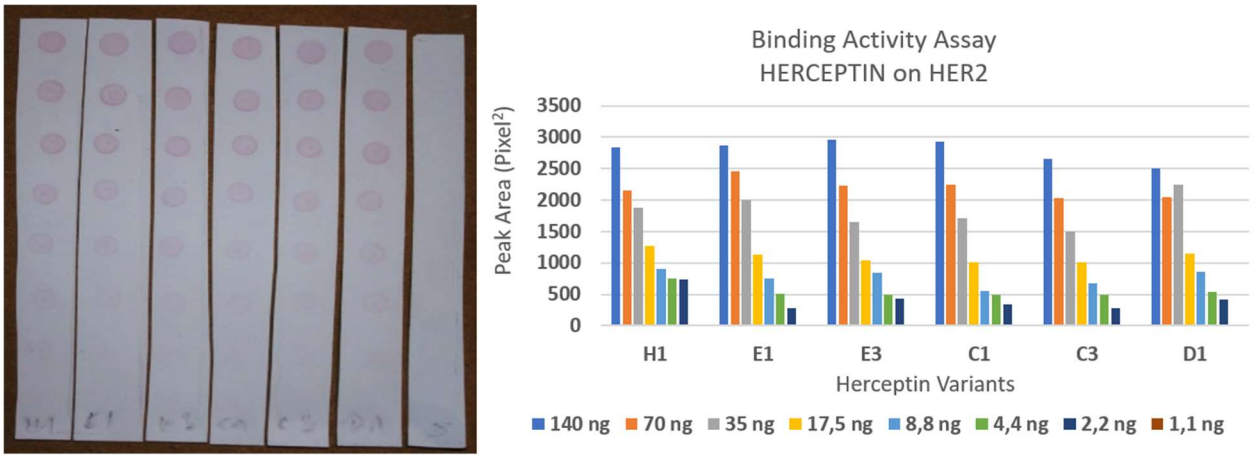


Figure 9: Dot blot activity assay of Herceptin samples, as shown in Figure 7. The test strips were dried, photographed and analyzed with an image analysis tool (here ImageJ).

PIA-PINK-SPEED Quick Guide

Immunostaining of ANTIBODIES in Dot Blot Assays

Preparatory Work

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

Immunostaining with PIA-PINK-SPEED after Protein Spotting on the Membrane

- Place the blot membrane in a clean dish and incubate covered with PIA-PINK-BLOCK Blocking Solution for at least 2 minutes with shaking. Discard the solution.
- Cover the blot membrane with PIA-PINK-SPEED Staining Solution and incubate for 15 minutes 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-SPEED Quick Guide

Immunostaining of ANTIGENES in Dot Blot Assays

Preparatory Work

- Fill the required volumes of PIA-PINK-SPEED 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-SPEED Staining Solution and allow it to hybridize for one hour. Ideal hybrids require 0.2 µg of primary antibody per mL of PIA-PINK-SPEED Staining Solution.

Immunostaining with PIA-PINK-SPEED-Hybrid after Protein Spotting on the Membrane

- Place the blot membrane in a clean dish and incubate covered with PIA-PINK-BLOCK Blocking Solution for at least 2 minutes with shaking. Discard the solution.
- Cover the blot membrane with PIA-PINK-SPEED primary antibody hybrid solution and incubate for 15 minutes 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-SPEED Immunoassays

These instructions explain how to use PIA-PINK-SPEED in a dot blot immunoassay for an NC membrane (12.5 x 8.0 cm) with antigen samples from a 96-well plate and a standard series (104 samples in total). To cover the membrane, 10 mL of each reagent is needed. 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-SPEED 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-SEED 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-SPEED. Then, add 2.0 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.

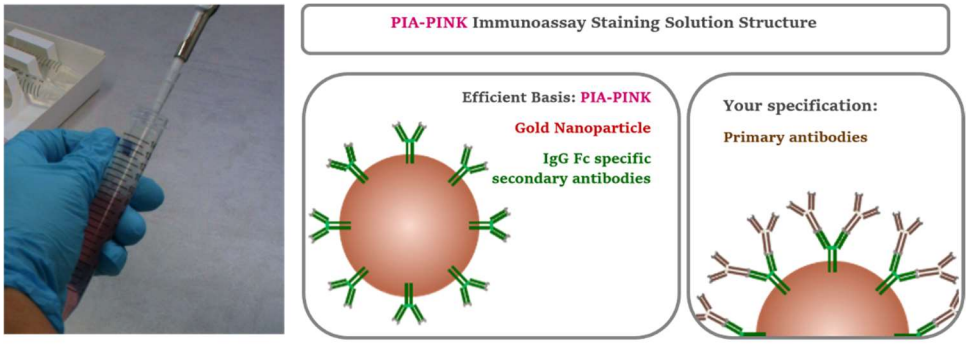


Figure 10: Preparation of the hybrid solution by adding the primary antibody to PIA-PINK-SPEED.

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 0.2 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 1/8 of the dilution recommended by the manufacturer for dot blots. Ideally use affinity purified antibodies.

Dot Blot

The dot blot is the optimal application for PIA-PINK-SPEED due to its rapid sample application and analysis, making it ideal for evaluating unclear samples. Please refer to the application examples above describing the identification of fractions during protein purification, screening of expression conditions, and membrane-bound activity test. 1.5 µL to 5.0 µL spot size is ideal to see the signal. Both NC and PVDF membranes are equally suitable for PIA-PINK immunostaining.

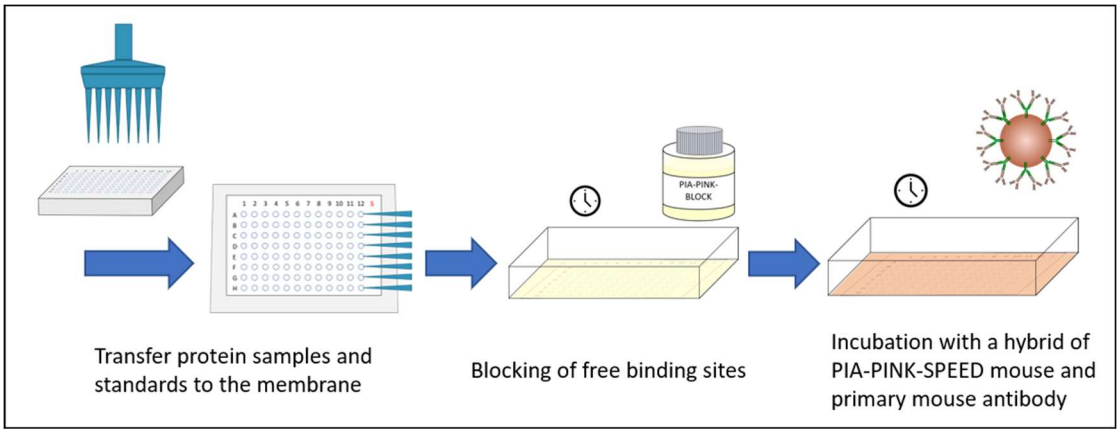


Figure 11: General procedure of a dot blot screening assay with PIA-PINK-SPEED primary antibody hybrid. ***Sample Application***

1. Sample spotting

To perform a dot blot analysis of screening samples in a 96-well format, prepare appropriately sized membranes. Mark the positions of the 96 samples and 8 standards with a soft pencil. Apply 1.5 to 5.0 µL of sample to each position. If larger volumes are needed, use slot blot devices. Apply a series of standards in the same volume next to the samples. (Figure 12)

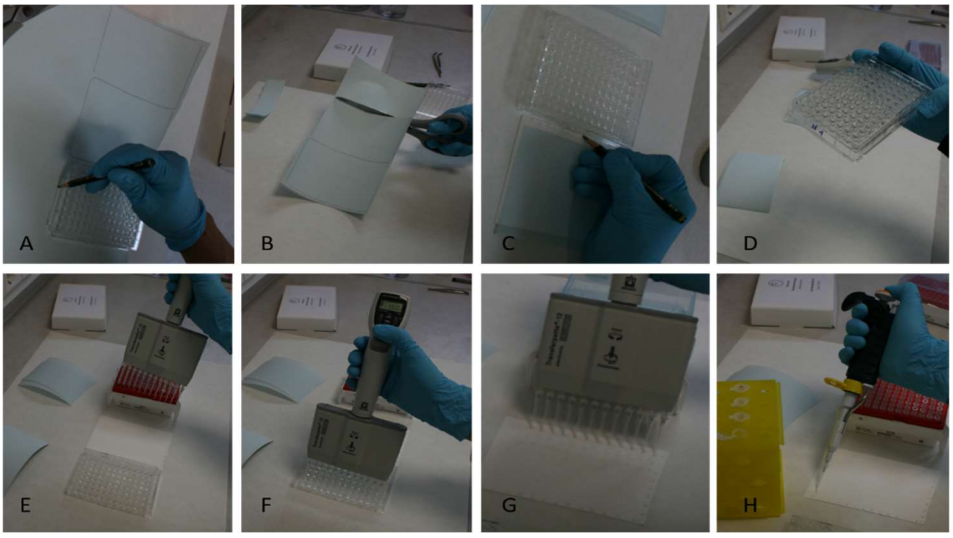


Figure 12: Preparation of cellulose nitrate membranes (NC) for dot blot analysis with PIA-PINK. A blank 96-well plate (12.5 x 8.0 cm) is used here as a format template. The outline is transferred to the protective paper of the NC (A), the NC is cut to size (B), the positions are marked with a soft pencil, starting offset to the left in order to obtain a 13th row for the standard (C). The sample plate is prepared (D), 1.5 µL to 5.0 µL of sample is applied (E-G). Dilutions of the standard series are applied (H).

2. Blocking of Free Protein Binding Sites on the Membrane

After applying and drying the 104 samples, place the Dot blot membrane in a clean dish and cover it with 10 mL of PIA-PINK-BLOCK. To saturate the free protein binding sites, incubate for at least 2 minutes with vigorous shaking. Then, discard the blocking solution. (Figure 13)

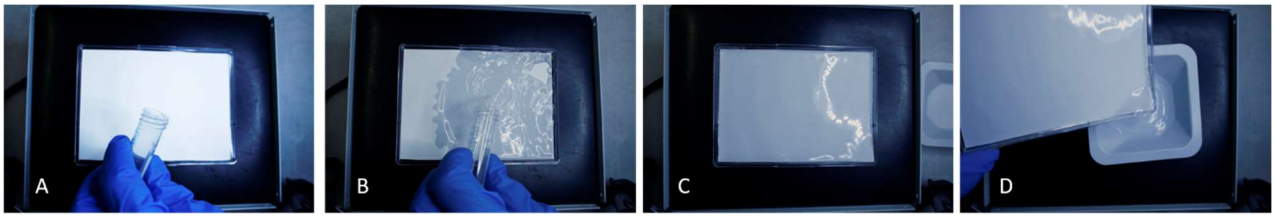


Figure 13: After sample application, the dot blot membrane is placed in a clean dish (A), covered with the appropriate amount of PIA-PINK-BLOCK Blocking Solution (B) and incubated for at least 2 minutes with shaking (C). The blocking solution is then discarded (D).

3. Immunostaining and Detection

Cover the nitrocellulose (NC) membrane with 10 mL of PIA-PINK-SPEED primary antibody hybrid and incubate for 15 minutes 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 spot. Slower shaking will prolong the incubation time. The degree of enrichment depends on the epitope concentration and the incubation time. (Figure 13) For instance, immunostaining of 30 pmol of antigen (equivalent to 1 µg of 30 kDa) becomes visible within 5 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 spot using PIA-PINK-SPEED is 15 minutes. The detection limit for PIA-PINK is approximately 30 fmol of antigen (equivalent to 1 ng of a 30 kDa protein) per spot. Color intensity can be observed and photographed during incubation. Immunostaining can be stopped or interrupted at any time and resumed if necessary. Generally, 15 minutes of incubation with PIA-PINK-SPEED 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.

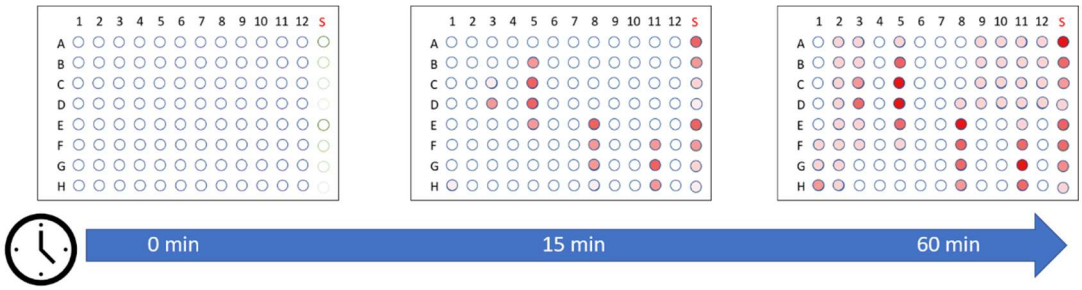


Figure 14: The intensity of the immunostaining with PIA-PINK-SPEED increases until the detection limit of approx. 30 fmol/µL is reached. For spots with an antigen concentration of 1 pmol/µL, a 15-minute incubation time is usually sufficient.

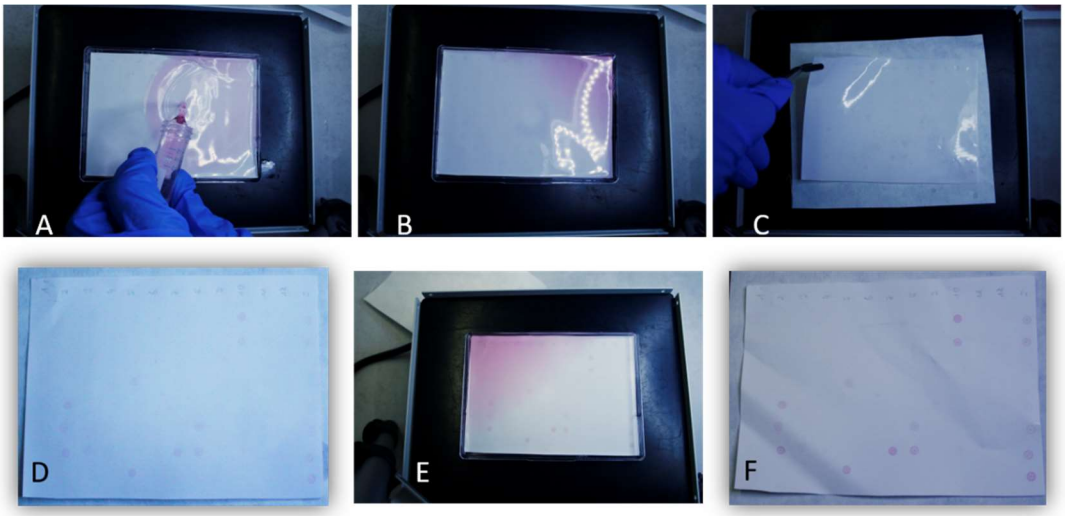


Figure 15: After blocking, the dot blot membrane is covered with the bottled PIA-PINK Staining Solution, which may be hybridized with the primary antibody (A), and incubated with shaking (B). The incubation can be interrupted at any time. The membrane is removed from the solution, dried, analyzed and photographed or scanned. Membrane after 15-minute incubation (D). Incubation can be continued at any time (E). Membrane after 30-minute incubation (F). The intensity of the immune stained spots increases with the incubation time until saturation.

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.

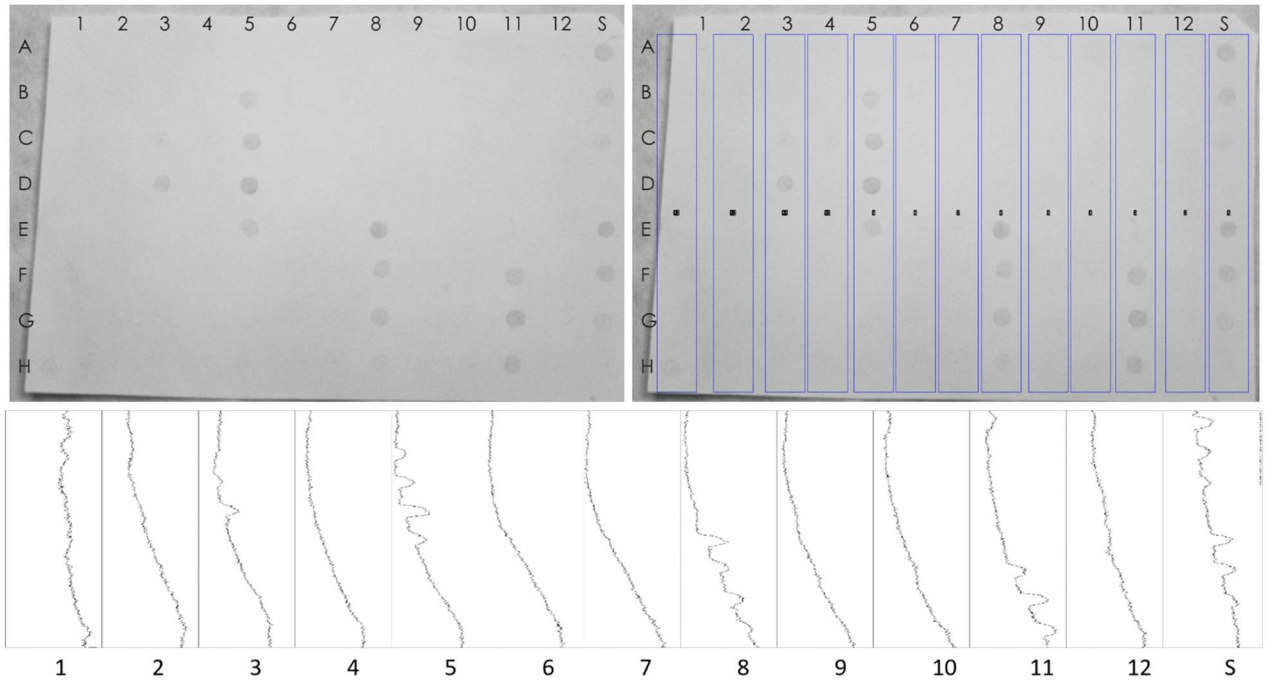
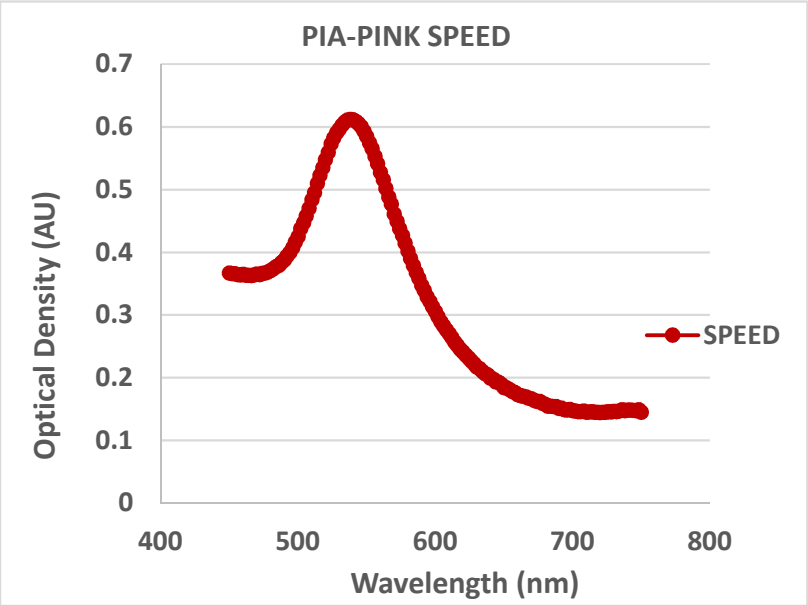


Figure 16: Example of quantification with ImageJ. Top left: Jpg file of the photographed dot blot from Figure 5 in 8-bit grayscale format. Top right: Selection of the columns (lanes). Bottom: Lineplots of the pixel intensities of the respective lanes. After compensating for the baseline shift caused by shadows on the photo, the peak area is determined and used for quantitative evaluation as described in Figure 6.

Product Characteristics

Article	**PIA-PINK-SPEED 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 # 103-31 Goat-Anti Rabbit IgG (Fc) # 104-31 Goat-Anti Mouse IgG (Fc) # 105-31 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) 0,6. 
Other Components	Water, BSA, PBS, Tween 20, Citrate @ pH 7.4
Recommended Applications	Dot Blot, Slot-Blot
Further Applications	Western-Blot, Vertical-Flow-Assay, Lateral-Flow-Assay, Electron microscopy, Darkfield microscopy

Product Form	This package includes two 50 mL bottles of ready-to-use solutions: PIA-PINK-SPEED 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 0.2 µg of affinity-purified IgG primary antibody per mL of PIA-PINK-SPEED 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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