BioTeZ

Kit for TA-PAS Labelling System

For immobilization, detection and enrichment of biomolecules in various applications

Alternative to or in combination with Biotin/Streptavidin-System

Instructions

Reagents for coupling a TA-Label to target molecules and to detect with specific PAS-Antibody

Product Code: BTTAP-001

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Note: Read the instructions carefully before using the reagents!

Intended Use

The TA-PAS System is a fast two component protein-ligand interaction system. One component is the small synthetic label called "TA-Label" (<1000 Da). The second component is the "PAS-Antibody" which binds the "TA-Label" with high specificity and efficiency.

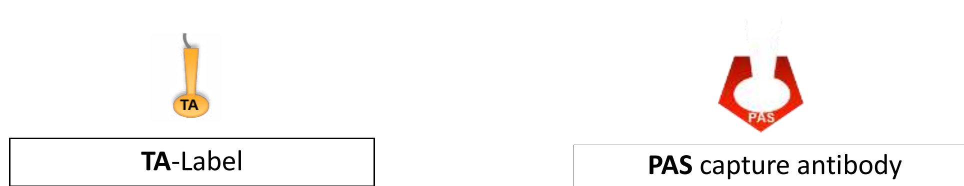

Figure 1: TA-PAS system: Two component protein-ligand interaction system consisting of TA-Label and PAS-Antibody (capture antibody). It can be flexibly combined with various target molecules and detection systems (fluorescent, enzymatic, luminescent).

The TA-PAS System supports a large number of applications including immobilization, enrichment or detection of antibodies, other proteins, primers and other nucleic acids. The TA-PAS System is a simple yet elegant system to link TA labelled molecules in assays by exploiting the high affinity to PAS capture antibody.

Applications

- Fast binding universal detection system, e.g. lateral flow assay (**Figure 2**)
- ELISA (**Figure 3**), it can be used as detection or coating component
- Immunohistochemistry (IHC)
- Western-, Southern- und Northern Blot
- Immunoaffinity chromatography
- Cell surface labelling etc.
- Alternative to Biotin-Streptavidin or in combination with Biotin-Streptavidin (**Figure 3**)
- TA-PAS with double labelled molecules like primers, antibodies, proteins (**Figure 2**)
- Immunoprecipitation: Immobilization of target molecules from cell culture or other biological samples
- Target molecule enrichment

Application Example: Lateral Flow Test Control Line

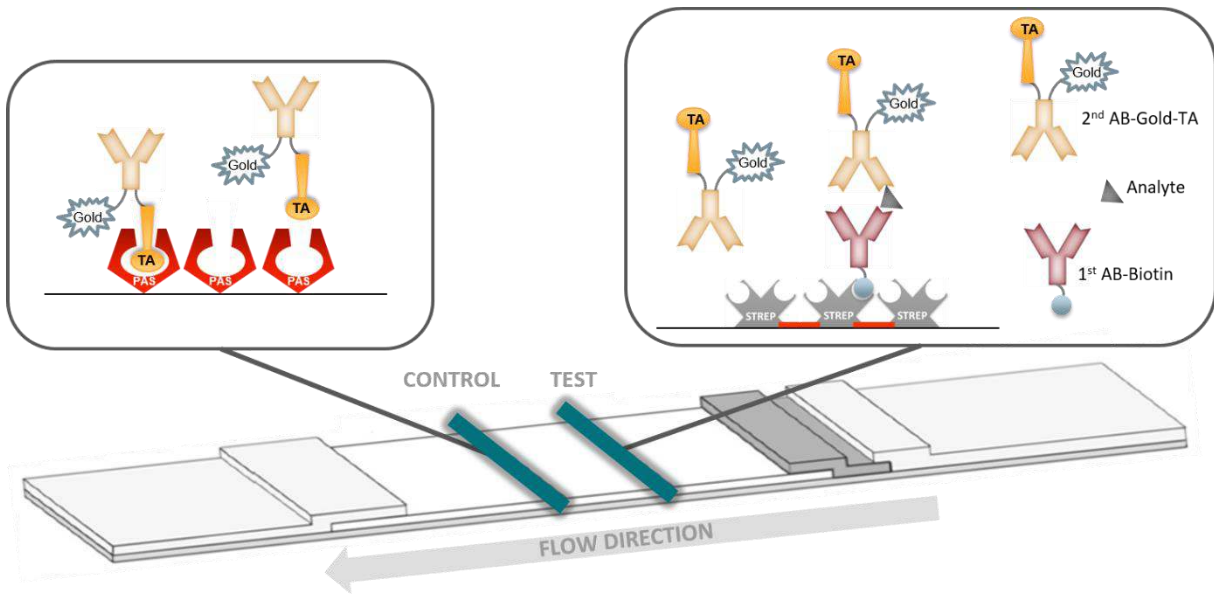

Figure 2: Application of TA-PAS system in lateral flow in combination with a double labelling strategy (antibody conjugated with Gold nanoparticles and TA-Label) for immobilization at control line and streptavidin*/biotin system for test line.

*For better signals using of BioTeZ Polystreptavidin is recommended.

Application Example: Detection with ELISA

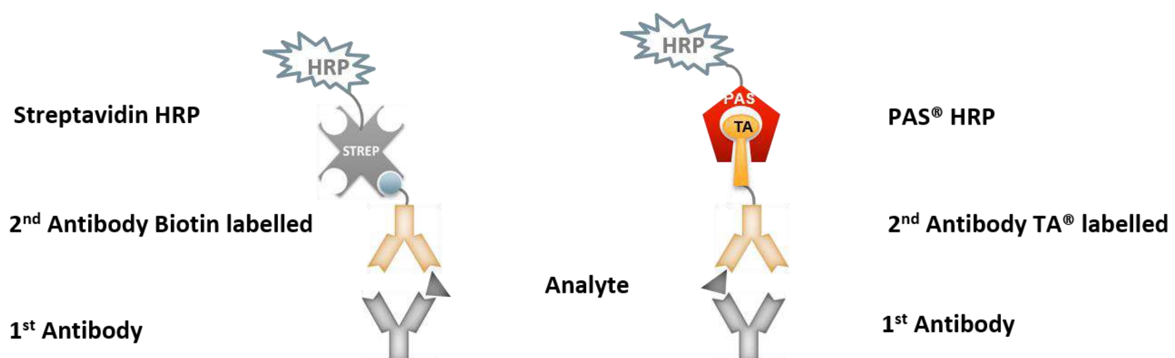

Figure 3: Comparison of Biotin-Streptavidin System and TA-PAS System in ELISA. Testing BioTeZ Poly-HRP is recommended.

Advantages

- The TA-PAS system allows a flexible, simple use in many applications
- Improvement of signal-noise ratio
- High affinity binding between TA-Label and PAS-Antibody (capture antibody)
- **Fast Binding**
- Low cross-reactivity
- Low non-specific binding
- **TA-PAS interaction is artificial and not present in nature**
- **No problems with samples or proteins with biotin** (e.g. Carboxylases, Histones)
- pH-stable
- Long shelf life time
- Reproducible results
- No problems with PAS coating followed by blocking with serum
- No problems with **double labelling** strategies for **lateral flow test** (figure 2)

Application Example: Immobilisation of TA-labelled Molecules

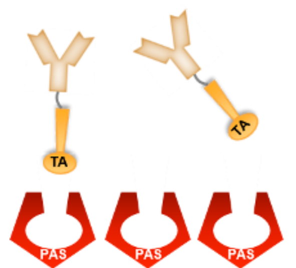

Figure 4: PAS is bound to a solid phase and immobilizes TA-labelled antibody

Description

The TA-PAS labelling kit allows antibodies, proteins, primer etc. to be covalently labelled with the TA-Label quickly and easily. The pre-activated TA-Label is designated for the coupling reaction with target molecules through the reaction with primary amino groups.

BioTeZ TA-PAS labelling kit includes the PAS-Antibody (capture antibody), all necessary reagents to label the target molecule with the TA-Labels and a clean-up column for purifying the coupling product (separation of unbound labels).

Double Labelling with TA-PAS™

Generally the TA-Label is fully compatible with other labels and should work well. For double labelling TA-Label and gold nanoparticles it is recommended to do TA-labelling procedure first. After this procedure carry out your gold labelling protocol or gold labelling kit instruction as usual.

Storage and Components

The **TA-PAS kit** is shipped at 2-8°C
Store the **pre-activated TA-Label** at -20 °C. All other components of the kit should be stored at 2-8°C.

Table 1: Overview of kit contents and storage conditions

Components	Colour of top cap	Store at	Quantity
Ultrapure Water	yellow	2 - 8 °C	20 ml
Coupling Buffer	green	2 - 8 °C	50 µl
Pre-activated TA-Label*	red	-20 °C	1 Aliquot
10x Purification Buffer	black	2 - 8 °C	2 ml
Clean-up column BTZ2	-	2 - 8 °C	1 x
PAS Antibody (contains sodium azide)	blue	2 - 8 °C**	1 x 0.2 mg

** The TA-Label is especially sensitive to moisture. Avoid contact with moisture.*
*** short term storage at 2-8°C, long term storage at -20°C*

Required equipment and materials not included in the kit:

- Thermoshaker
- Vortex Mixer
- Stand with clamp
- Beaker/Container
- Spectrophotometer
- Pipette

Considerations prior to Coupling/Labelling

- Target to be labelled must have free amino groups!
- Target must be purified. Recommended is storage in Purification Buffer pH 7.4.
- We recommend a start concentration of antibody >1 mg/ml; as this allows potentially interfering substances in the antibody preparation to be diluted out.
- No amino acids e.g. glycine!
- No primary amines e.g. **Tris!**
- No thiols e.g. DTT, cysteine!
- Some enzymes can lose their activity because of labelling

Coupling protocol for Antibodies/other Proteins

A) *Preparation of the column (hands-on time 50 min)*

- Attach the column to the stand with a clamp
- Dilute 1.5 ml **10× Purification Buffer** (**black** lid) with 13.5 ml **Ultrapure Water** (**yellow** lid)
- Put a waste container under the column and equilibrate the column with 10 × 1 ml **1× Purification Buffer**
- Discard the flow
- Till use, keep the column closed

B) *Coupling of Antibody/other Protein* (hands-on time 30 min)

- Dilute 100 µg antibody/667 pmol of other protein with **Ultrapure Water** (**yellow** lid) to 180 µl (final conc. antibody = 0.56 mg/ml; other protein = 3.71 pmol/µl)
- Add 20 µl **Coupling Buffer** (**green** lid) to antibody/protein solution – total volume 200 µl
- Equilibrate the **Pre-activated TA-Label (red** lid**)** to room temperature before use
- Transfer the whole 200 µl antibody/protein solution to the tube with **Pre-activated TA-Label** (**red** lid). ***Avoid long and unnecessary opening of the lid of TA-Label.***
- Mix until the solution is evenly coloured (vortex mixer).
- Incubate 20 min at 18-20 °C in an incubator or thermoshaker without shaking
- Centrifuge shortly to collect the fluid

C) *Purification of the labelled Antibody/Protein (hands-on time 30 min)*

- Transfer the 200 µl coloured solution onto the column
- When the solution has completely entered the column then add 80 µl **1× Purification Buffer** onto the column
- Discard the flow
- Change the container and put a 1.5 ml tube under the column
- Elute the labelled antibody/protein solution with 420 µl **1× Purification Buffer** into the 1.5 ml tube

D) *Quantification and Calculation for Antibodies*

Determination of the protein concentration by UV:
Determine the absorbance of the labelling product at 278 nm and 548 nm. For this purpose place a sample in a quartz cuvette. Please consider the measuring range of your UV spectrophotometer.

Determination of *Antibody concentration in clear solutions*

$$c_{Ab} = \frac{Abs_{278} - F \cdot Abs_{548}}{a\ Ab}$$

Remarks: In addition to the absorption at 548 nm TA has also an absorbance at 278 nm. For the calculation of the protein concentration, a correction of the measured absorbance at 278 nm is therefore required (see factor F).

c_{Ab} – Concentration of antibody [mg/ml]
Abs_{278} – Absorption at 278 nm
F – Correction factor absorption of TA at 278 nm = 0.3
Abs_{548} – Absorption at 548 nm
α_{Ab} – Extinction coefficient of antibody =1.38

The antibody yield is typically about 80 %.

Degree of labelling (DOL) in clear solutions

The degree of labelling (DOL) is the average number of TA-molecules coupled to a protein (e.g. an antibody). The DOL can be determined from the absorption spectrum of the labelled antibody.

$$DOL = \frac{MW_{Ab} \cdot Abs_{548}}{c_{Ab} \cdot \varepsilon TA}$$

MW_{Ab} – Molecular weight of the antibody =150,000 [g/mol]
Abs_{548} – Absorption at 548 nm
c_{Ab} – Concentration of the antibody [mg/ml]
ε – molar extinction coefficient TA=78,000 [M^{-1} cm^{-1}]

Attention: Turbid solutions might give wrong results, especially in case of low antibody concentrations.

E) *Quantification and Calculation for Proteins*

Determination of the protein concentration by UV:
Determine the absorbance of the labelling product at 280 nm and 548 nm. For this purpose place a sample in a quartz cuvette. Please consider the measuring range of your UV spectrophotometer.

Determination of protein concentration *<u>in clear solutions</u>*

$$c_{protein} = \frac{Ab_{280} - F \cdot Abs_{548}}{a_{protein}}$$

$c_{protein}$ – Concentration of protein [mg/ml]
Abs_{280} – Absorption at 280 nm
F – Correction factor absorption of TA at 280 nm = 0.3
Abs_{548} – Absorption at 548 nm
$\alpha_{protein}$ – Extinction coefficient of protein ($\alpha = \varepsilon/MW$)
for determination of ε see https://web.expasy.org/protparam/

Remarks: In addition to the absorption at 548 nm TA has also an absorbance at 280 nm. For the calculation of the protein concentration, a correction of the measured absorbance at 280 nm is therefore required (see factor F).

The yield is typically about 80 %.

Degree of labelling (DOL) *<u>in clear solutions</u>*

The degree of labelling (DOL) is the average number of TA-molecules coupled to a protein. The DOL can be determined from the absorption spectrum of the labelled protein.

$$DOL = \frac{MW_{protein} \cdot Abs_{548}}{c_{protein} \cdot \varepsilon TA}$$

$MW_{protein}$ – Molecular weight of the protein [g/mol]
Abs_{548} – Absorption at 548 nm
$c_{protein}$ – Concentration of the protein [mg/ml]
ε – Molar extinction coefficient TA = 78,000 [M^{-1} cm^{-1}]

Attention: Turbid solutions might give wrong results, especially in case of low antibody concentrations.

F) *<u>Storage of Conjugate</u>*

Store the conjugate just like the unlabeled antibody/protein. We recommend dividing the solution into small aliquots. Avoid repeated freezing and thawing!

PAS-Antibody

Solute the lyophilized PAS-Antibody in water/buffer for your application.
The lyophilisate contains PBS salts and sodium azide. The PAS-Antibody captures the TA-labelled antibodies/other proteins.
Each laboratory should determine the optimum working dilution for use in its particular application.

Troubleshooting

Low concentration of labelled antibody or protein	• Wrong concentration of antibody/target • Unpurified antibody or contamination with other proteins • Interrupted cold chain of Labelling Kit or storage conditions are wrong • Expiry Date not valid
High concentration of labelled antibody or protein	• Wrong concentration of antibody/target
Non-labelling	• storage conditions are wrong • Interrupted cold chain of Labelling Kit • Buffer contains amine; thiols (see considerations prior to coupling/labelling)
Inefficient Labelling (DOL<1.2)	• Protein solutions containing primary amines dramatically decrease labelling efficiency. Make sure that your protein is extensively dialyzed in case it has been in contact with amine-containing substances. • The pH of your protein solution! The primary amino groups of the protein must not be protonated to be reactive thus, the pH of the protein solution has to be sufficiently high.
Overlabelling (DOL>3.5)	• a high number of lysines on the surface of your protein

Related products

Product	Cat. No.	Package Size
BioTeZ Peroxidase Labelling Kit Preparation of HRP labels using highly activated Poly-HRP	BTHRPK-03 BTHRPK-05	For 3 labels For 5 labels
Biotin Labelling Kit Reagents for the biotinylation of 1 – 10 mg antibody / 1-5 mg other biomolecules	BTBIOK-05 BTBIOK-10	For 5 labels For 10 labels
TA-PAS Labelling Kit (for immobilization and enrichment of biomolecules) Alternative to or in combination with Biotin/Streptavidin	BTTAP-001	For 1 label
PAS-Gold PAS capture antibody gold labelled (40nm)	20 500 005 20 500 020	0.5 ml 2.0 ml
PAS-HRP PAS capture antibody HRP labelled	20 600 001	100 µg
PAS-Poly-HRP PAS capture antibody Poly-HRP labelled (40nm)	20 700 001	100 µg
Polystreptavidin E (Polystrept E) Very high Biotin binding capacity; optimized for using biotin-streptavidin in combination with TA-PAS system in lateral flow tests	10 121 010 10 121 010 10 121 010	1 mg 5 mg 10 mg
Recombinant Streptavidin, FITC-labelled	20 400 001	100 µg
Recombinant Streptavidin, Poly-HRP-labelled	20 201 001	100 µg
Recombinant Streptavidin	10 110 010 10 110 050 10 110 100	1 mg lyophilized 5 mg lyophilized 10 mg lyophilized
Polystreptavidin R (Polystrept R) Very high Biotin binding capacity	10 120 010 10 120 050 10 120 100	1 mg 5 mg 10 mg
Streptavidin-coated 8-well strip plate, C well, clear Polystyrene	F8PS-CL-SC	5 plates
Polystreptavidin-coated 8-well strip plate C well, clear Polystyrene	F8PS-CL-MC	5 plates
Labelled Streptavidin Poly HRP, HRP, Gold, FITC, EU, Carbon Black		
Streptavidin coating kits	**Cat. No.**	**Package Size**
BioTeZ Streptavidin Coating Kit For Streptavidin Coating on beads, chips, membranes etc.	BTCK-SC0125 BTCK-SC0500	1 Kit/ 125 ml solution 1 Kit/ 500 ml solution
BioTeZ Polystreptavidin R Coating Kit For Polystreptavidin R Coating on beads, chips, membranes etc.	BTCK-MC0125 BTCK-MC0500	1 Kit/ 125 ml solution 1 Kit/ 500 ml solution
BioTeZ Polystreptavidin R Coating Kit Glass For Polystreptavidin R Coating on glass	BTCKG-MC0125 BTCKG-MC0500	1 Kit/ 125 ml solution 1 Kit/ 500 ml solution

Important points and precautions

1. The manufacturer assumes no liability and indicates that the user is solely responsible for the consequences of any alterations made, for non-observance of instructions or for performing the coating procedure without paying due attention.
2. The equipment used must be maintained in accordance with the manufacturers' instructions and any applicable guidelines. Before equipment is used it should be checked for fault-free operation.
3. Excess reagents that have exceeded their expiry date/lifetime should be disposed of correctly. You should observe the regulations that apply to you.
4. The reagents may only be carried out by trained specialists. The production steps are validated for use at the indicated temperature. Deviations in the climatic conditions can negatively influence the results.
5. Ensure that the materials, equipment and reagents are clean, paying particular attention e.g. to vessels and pipette tips.
6. Observe general health and safety regulations.
7. Follow the instructions for this test very closely.
8. When disposing of the reagents, observe any potential harm they may cause to the environment. Observe the regulations that apply to you.
9. Observe safety regulations, e.g. do not eat, drink or smoke in the workplace; keep materials and reagents away from foods and feeding stuffs; wear protective clothing (lab coat, safety glasses and gloves).
10. Never carry out pipetting operations using the mouth; always use suitable equipment or devices.

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