Streptavidin-AP conjugate

Solution, stabilized
Cat. No. 11 089 161 001
Store at +2 to +8°C

Streptavidin-β-Gal conjugate

Lyophilizate, stabilized
Cat. No. 11 112 481 001
Store at −15 to −25°C

Strepavidin-POD conjugate

Lyophilizate, stabilized
Cat. No. 11 089 153 001
Store at +2 to +8°C

Version 21
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1. What this Product Does

Streptavidin-AP Conjugate

Preparation

Streptavidin from *E. coli* K12 was coupled to alkaline phosphatase (AP) from calf intestine via heterobifunctional linkers. This conjugate was purified using column chromatography. The final preparation is stabilized in 30 mM triethanolamine buffer, pH 7.6, 0.5% bovine serum albumin (w/v), 0.01% 2-methylisothiazolone (w/v).

Stability

The concentrated stock solution is stable when stored at **+2 to +8°C** until the expiration date printed on the label. **Do not freeze**!

Recommended Concentration

50 - 1,000 mU/ml, *i.e.,* the stock solution can be diluted:

ELISA:	1:5,000 - 1:20,000	= 25,000 - 100,000 tests
Immunohistochemistry:	1:1,000 - 1:4,000	= 20,000 - 80,000 sections
Immunoblotting:	1:2,000 - 1:5,000	= 50 – 125 protein blots, each 100 cm^2

Streptavidin-β-Gal Conjugate

Preparation

Streptavidin from *E. coli* K12 was coupled to b-galactosidase (β-Gal) from *E. coli* via heterobifunctional linkers. This conjugate was purified using column chromatography. The final preparation is stabilized in 10 mM potassium phosphate buffer, 25 mM NaCl, pH 7.2, 0.5% bovine serum albumin (w/v), 0.01% 2-methylisothiazolone (w/v) and lyophilized. Dissolving the lyophilizate in 1 ml redist. water results in a concentration of 500 U conjugate/ml.

Stability

The lyophilized conjugate is stable when stored dry at −15 to −25°C until the expiration date printed on the label. The reconstituted solution is stable for up to 4 weeks at +2 to +8°C. The solution can be stored in aliquots at −15 to −25°C until the expiration date printed on the label. Avoid repeated freezing and thawing.

Recommended Concentration

100 – 500 mU/ml, i.e. the stock solution can be diluted:

ELISA:	1:1,000 – 1:5,000	= 5,000 – 25,000 tests
Immunohistochemistry:	1:1,000 – 1:2,000	= 20,000 – 40,000 sections
Immunoblotting:	1:1,000 – 1:2,000	= 25 – 50 protein blots, each 100 cm2

Streptavidin-POD Conjugate

Preparation

Streptavidin from *E. coli* K12 was coupled to β-peroxidase (POD) from horse radish using the periodate-method (1). This conjugate was purified using column chromatography. The final preparation is stabilized in 60 mM Tris-Hepes buffer, pH 7.2, 0.4% bovine immunoglobulin (w/v), 0.01% 2-methylisothiazolone (w/v). Dissolving the lyophilizate in 1 ml redist. water results in a concentration of 500 U conjugate/ml.

Stability

The lyophilized conjugate is stable when stored at **+2 to +8°C** until the expiration date printed on the label. The reconstituted solution is stable for up to 6 months at +2 to +8°C. **Do not freeze**!

Recommended Concentration

5-500 mU/ml, i.e., the stock solution can be diluted:

ELISA:	1:10,000 – 1:100,000	= 50,000 – 500,000 tests
Immunohistochemistry:	1:1,000 – 1:2,000	= 20,000 – 40,000 sections
Immunoblotting:	1:2,000 – 1:5,000	= 50 – 125 protein blots, each 100 cm^2

The performance of the conjugates are analyzed in ELISA and on sections.

Application

The conjugates are used for the detection of biotin-labeled substances (*e.g.,* biotinylated antibodies) in several immunological detection systems (2,3) such as ELISA (4, 5), immunohistochemistry and immunocytochemistry (6, 7), immunoblotting (8, 9) and also screening of monoclonal antibodies.

The user can either purchase or synthesize biotinylated products (*e.g.,* antibodies or lectins). Biotin can be easily conjugated to several substances (*e.g.,* Amino, carboxyl, aldehyde, thiol groups and nucleic acids).

A description of these coupling procedures is available upon request.

Alkaline Phosphatase

Alkaline phosphatase is often used as a label in enzyme immunoassays. AP converts the sensitive substrate X-phosphate/NBT into a stable, water insoluble product.

β-Galactosidase

As β-galactosidase is not present in eukaryotes it is used as a marker enzyme in eukaryotic systems where endogeneous enzyme activities may interfere with AP assays. The recommended substrate for β-Gal assays in immunoblotting applications is X-gal, a colored substrate analogous to X-phosphate. Fluorescent β-Gal substrates can also be used for very sensitive ELISAs (10).

Peroxidase

Shows high sensitivity as a marker enzyme in ELISA systems. However, if POD is used on tissue sections, endogenous peroxidase may interfere with the assays.

General Information

U refers to the enzyme unit appropriate for AP, β-Gal or POD.
Biotin (vitamin H) is a readily water-soluble substance (M_r 244 Da) that binds with one of the highest naturally known affinities (10^{-15} mol^{-1}) to avidin (11), a glycoprotein (M_r 66 kDa, pI 10) from egg white. It also binds to streptavidin (12), a non-glycosylated protein (M_r 60 kDa, pI 7.2/7.4) from the bacterium *Streptomyces avidinii*. Each of the proteins has four binding sites for biotin. The binding is almost irreversible (cf. antigen/antibody: 10^{-7} to 10^{-11} mol^{-1}).

Both proteins can be conjugated with numerous signal providing substrates, *e.g.,* gold, enzymes and fluorescent dyes, so that the most favorable assay system can be designed.

Another assay variation consists of labeling the signal-providing enzymes with biotin and then detecting *e.g.,*, the Ig-biotin conjugate either

a) with a complex of avidin/streptavidin + biotin-enzyme **or**

b) by initial incubation with avidin/streptavidin and subsequent addition of the biotinylated enzyme.

An isoelectric point in the neutral range and the absence of carbohydrate fractions make streptavidin preferable to avidin, for assays in a neutral pH range. Carbohydrate-lectin interactions do not interfere with streptavidin assays, allowing them to have low "backgrounds".

Instructions for the Assay Procedure

- ELISA
- Immunoblotting
- Immunohistochemistry

General

The following data are only intended as guidelines and as aids to establishing an immunotest. The performance of the system can be adjusted to individual assay requirements by variation of such parameters as the quality of the reagents, concentrations, buffers, incubation times and temperature.

Reagents

Only analytical quality reagents should be used.

Solid Phases for ELISA and Immunoblotting

Microtiter Plates

Choice of plate material is critical for ELISA tests. Use only plates which show high protein and peptide binding capacity, even in the presence of detergents, and which show high homogeneity within each plate and between plates.

Membranes

There are numerous different types of membranes on the market for immunoblotting. The most frequently used for adsorption is nitrocellulose (NC), but nylon- and PVDF-membranes can also be used. For covalent binding activated cellulose paper can be used. DEAE paper can be used for ionic binding.
(PVDF = polyvinylidene difluoride;
DEAE = diethylaminoethyl).

Tissue Sections

Frozen Sections

Ideal frozen sections (4 – 5 mm) are obtained from shock frozen tissue samples. Air dry the frozen sections and fixed them with acetone for 10 min at −15 to −25°C. Remove excess acetone by drying or by washing with PBS.

Paraffin sections

Dewax formalin-fixed, paraffin-embedded tissue sections by extracting them twice with xylene, A.R. (10 min per extraction). Wash the xylene out with steadily decreasing concentrations of ethanol (down to 70%). Wash the sections in redist. water and equilibrate in PBS. Depending on the type of primary antibody, treatment with proteases, *e.g.,* 0.1% trypsin* (w/v), or pepsin*, is recommended.

Cell Preparations

Cell Suspensions

Adjust cells to 10^6 – 10^7/ml in medium and add the primary antibody to the untreated sample. If interfering substances are present in the medium centrifuge the cell suspension for 20 min at +15 to +25°C (400 – 600 × g), wash with PBS and take the cells up in the desired medium, *e.g.,* PBS or PBS with 1% bovine serum albumin (w/v), or in PBS with 0.1% bovine-Ig (w/v), for blocking of the Fc-receptors.

Cell Smears

Smear one drop of the sample, *e.g.,* blood, with a cover glass slide, let air-dry and fix with acetone, methanol or methanol/acetic acid [95%/5% (v/v)] for 10 min at −15 to −25°C. Wash out the fixative with PBS or let dry.

Cytospin Preparations

Centrifuge (ca. 100 × g) cell suspensions for 10 min at +15 to +25°C onto a glass slide using a Cytospin apparatus. Remove apparatus, suck off excess liquid, wash the cells for a short time in PBS and fix them in acetone or methanol. Wash the fixative off with PBS or let dry.

Cover Slip Preparations

Use forceps to pick up cover slips that contain cells, remove excess liquid carefully by suction, wash the cells for a short time in PBS and fix the cells in acetone or methanol for 10 min at −15 to −25°C. Wash the fixative off with PBS or let dry.

Tissue Culture Chamber/Slide

Remove liquid carefully by suction, wash the cells for a short time in PBS and fix the cells in acetone or methanol for 10 min at −15 to −25°C. Wash out the fixative with PBS or let dry.

Fixation on Terasaki Plates

Precoat the wells with 10 µl poly-L-lysine in PBS,
25 µg/ml, for 20 min at +15 to +25°C and wash the wells twice in PBS.
Pipette the cells (10^4-10^6/ml) suspended in PBS into the wells and centrifuge (100 – 200 × *g* for 15 min at +15 to +25°C. Remove the supernatant by suction, add 0.01% glutardialdehyde solution (v/v), and incubate for 5 min at +15 to +25°C.
Wash the wells once with PBS and block the binding sites with 1% bovine serum albumin (w/v) in PBS, for 20 min at +15 to +25°C.

Paraformaldehyde Fixation

(Alternative to acetone and methanol fixation of surface antigens on cells and in tissue sections)

3.7% paraformaldehyde, A.R. (v/v), 1 mM $CaCl_2$, 0.5 mM $MgCl_2$ in PBS, pH 7.4. Filter solution and cool to 0 to +2°C (ice-bath). Wash tissue sections or cells, respectively, on the slide twice with PBS and fix for 15 min at 0 to +2°C (ice-bath) in paraformaldehyde solution. Wash the preparation once with PBS and subsequently incubate with 0.1% Triton* X-100 (v/v), in PBS at +15 to +25°C (1 – 5 s for surface antigens, 5 min for permeation of the cell membrane). Wash the preparation three times with PBS and block nonspecific binding sites with fetal calf serum.

Buffers/Solutions

PBS

Phosphate buffered saline
10 – 20 mM potassium phosphate or sodium phosphate or potassium-sodium phosphate,
pH 7.2 – 7.4, 150 mM sodium chloride.

TBS

Tris-buffered saline
20 – 100 mM Tris-HCl, pH 7.5 – 8.0, 150 mM sodium chloride.

$NaHCO_3/Na_2CO_3$

50 mM sodium hydrogen carbonate/sodium carbonate, pH 9.6.

Solutions for Inhibition of Endogeneous Enzymes on Tissue Sections

For Alkaline Phosphatase

1 mM Levamisol, in PBS.

For Peroxidase (13)

- 0.074% HCl (v/v) in ethanol, A.R. (fixation step).
- 0.3 - 3% H_2O_2 (v/v) in PBS, incubate for 30 min at +15 to +25°C.
- 0.1% Phenylhydrazine (w/v) in PBS, incubate for 1 h at +37°C with freshly prepared solution.

Protein Additive/Blocking Reagents

Bovine serum albumin* (BSA), ovalbumin (OVA), gelatin, dried milk powder or animal sera correspon-ding to the secondary antibody are frequently used. Concentrations usually range from 0.2 to 2% (w/v). Add sera at a concentration of 10% (v/v). It should be noted that varying qualities of each of these materials are on the market.

Detergents

0.02 – 0.1% Tween* 20 (v/v), 0.02 – 0.1% Triton X-100* (v/v). Detergents are frequently used in ELISA, but in histochemistry detergents are generally omitted. Their use in immunoblotting is determined on a case to case basis.

Preservatives

It is best to add preservatives to the buffers and solutions in order to prevent microbial contamination. 0.1% sodium azide (w/v) is frequently used. Sodium azide inactivates peroxidase and so, in this case thymol (up to saturation point) is often used instead. An alternative to thymol would be Bromo-nitro-dioxane*.

Substrate Solutions

The substrates used depend on the application, *e.g.,* substrates with water-soluble products for microtiter-plate-ELISA, substrates with water-insoluble products for immunohistochemistry and immunoblotting.
The solutions should always be freshly prepared.

1. For Alkaline Phosphatase

pNPP
4-Nitrophenylphosphate* 10 mM pNPP in 1 M diethanolamine buffer, pH 9.8, 0.5 mM $MgCl_2$. The resulting product is yellow and soluble in water. Measurement at 405 nm.

Fast Red, TR salt/NABP
4-Chloro-2-methylbenzene diazonium chloride/naphthol-AS-BI phosphate 0.4 mM NABP, 38.8 mM Fast Red, TR salt in 100 mM Tris-HCl, pH 8.2. Dissolve the NABP first in dimethylsulfoxide and add Fast Red shortly before use. The resulting product is red and insoluble in water but soluble in ethanol.

Fast Red, TR salt/NAMP
4-Chloro-2-methylbenzene diazonium chloride/naphthol-AS-MX phosphate; 0.5 mM NAMP, 2.0 mM Fast Red, TR salt in 100 mM Tris-HCl, pH 8.2. Dissolve the NAMP first in dimethylsulfoxide and add Fast Red shortly before use. The resulting product is red and insoluble in water and ethanol.

New Fuchsin/NABP
New Fuchsin/naphthol-AS-BI phosphate 5.0 mM NABP, 0.05% New Fuchsin (w/v) in 200 mM Tris-HCl, pH 9.2, 6 mM sodium nitrite. Dissolve NABP first in dimethylsulfoxide and New Fuchsin in 2 M HCl. The resulting product is carmine-red and insoluble in water and ethanol.

X-phosphate/NBT
5-Bromo-4-chloro-3-indolyl phosphate*/nitro-blue tetrazolium chloride 0.38 mM X-phosphate, 0.41 mM NBT in 200 mM Tris-HCl, pH 9.5, 10 mM $MgCl_2$. Dissolve X-phosphate first in dimethylformamide and NBT in 70% dimethylformamide (w/v). The resulting product is blue and insoluble in water.

2. For β-Galactosidase

oNPG 2-Nitrophenyl-β-D-galactopyranoside*
3 mM oNPG in 10 mM K-phosphate, 150 mM NaCl, 10 mM $MgCl_2$, 100 mM 2-mercaptoethanol, pH 7.5. The resulting product is yellow and soluble in water. Measurement at 405 nm.

CPRG Chlorophenol red-β-D-galactopyranoside*
3.29 mM CPRG in 100 mM Hepes*, 150 mM NaCl, 2 mM $MgCl_2$, 0.1% Na-azide (w/v), 1% bovine serum albumin (w/v), pH 7.0. The resulting product is dark red and soluble in water. Sensitivity is 10 times higher than that of oNPG. Measurement at 574 or 578 nm.

Resorufin-gal
Resorufin-β-D-galactopyranoside*
0.2 mM Resorufin-gal in 10 mM K-phosphate, 150 mM NaCl, 2 mM $MgCl_2$, pH 7.5. The resulting product is magenta, fluoresces and is soluble in water. Excitation: 550 – 572 nm, Emission: 583 nm.

MUG 4-Methylumbelliferyl-β-D-galactopyranoside*
0.1 mM MUG in 10 mM K-phosphate, 150 mM NaCl, 2 mM $MgCl_2$, 0.1% bovine serum albumin (w/v), pH 7.0. The resulting product fluoresces and is soluble in water. Excitation: 340 nm, Emission: 440 nm.

X-gal 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside*
1.2 mM X-gal, 3 mM potassium hexacyanoferrate (II), 3 mM potassium hexacyanoferrate (III) in PBS, pH 7.4. Dissolve X-gal first in a little dimethylformamide. The resulting product is blue and insoluble in water and ethanol.

NABG/Pararosaniline
Naphthol-AS-BI-β-D-galactopyranoside/pararosaniline hexazonium salt 0.4 mM NABG, 0.14% pararosaniline (w/v), in 100 mM citrate buffer, pH 5.0. The resulting product is red and insoluble in water and ethanol.

Fast Garnet/BNG
o-Aminoazotoluene, diazonium salt / 6-bromo-2-naphthyl-β-D-galactopyranoside 0.55 mM Fast Garnet, 0.29 mM BNG, in PBS, pH 7.4. Dissolve Fast Garnet first in 200 mM $MgCl_2$ (in approx. 1/20 of the total volume) and dissolve BNG first in DMSO (in approx. 1/50 of the total volume). Add both of the solutions to PBS. The resulting product is red and insoluble in water.

3. For Peroxidase

ABTS 2,2'-Azino-di-[3-ethylbenzthiazoline sulfonate (6)]*
100 mg ABTS substrate in 3.25 mM sodium perborate, 39.8 mM citric acid, 60 mM disodium hydrogen phosphate, pH 4.4 - 4.5. The resulting product is green and soluble in water. Measurement at 405 nm.

TMB 3,3',5,5'-Tetramethylbenzidine*
0.42 mM TMB, 0.004% H_2O_2 (v/v), in 100 mM sodium acetate/citric acid, pH 4.9. Stop the reaction with 2 M H_2SO_4. The resulting product is at first blue and, after the reaction is stopped, yellow and soluble in water. Measurement at 450 nm.

oPD 1,2-Phenylenediamine
4 mM oPD, 0.004% H_2O_2 (v/v), in 50 mM sodium phosphate, 20 mM citric acid, pH 5.0. Stop the reaction with 100 mM H_2SO_4. The resulting product is at first yellow and, after the reaction is stopped, orange and soluble in water. Measurement at 492 nm.

DAB Diaminobenzidine
(3,4,3',4',-tetraaminobiphenyl) 1.39 mM DAB, 0.01% H_2O_2 (v/v), in 50 mM Tris-HCl, pH 7.3. The resulting product is brown and insoluble in water and ethanol.

AEC 3-Amino-9-ethyl carbazole
0.32 mM AEC, 0.002% H_2O_2 (v/v), in 50 mM Tris-HCl, pH 7.3. Dissolve the AEC first in a little dimethyl-sulfoxide. The resulting product is red and insoluble in water but soluble in ethanol.

CN 4-Chloro-1-naphtol
5.6 mM CN, 0.01% H_2O_2 (v/v), in 50 mM Tris-HCl, pH 7.4, 150 mM NaCl. Dissolve the CN first in a little methanol. The resulting product is bluish black and insoluble in water, but soluble in ethanol.

Procedures

The following tables give examples of procedures for:

ELISA (procedure *e.g.,* for hybridoma screening), Table 1

Immunoblotting, Table 2

Immunohistochemistry or immunocytochemistry, Table 3

Tab. 1: Procedure for ELISA

	Procedure/ Incubation	Reagent	Volume µl	Reagent concentration µg/ml	Buffer	pH	Detergent	Protein addition	Incubation temperature	Incubation time
Step 1	Coating of microtiter plates	antigen	50 – 250	0.2 – 5	$NaHCO_3$/ Na_2CO_3 PBS/TBS	9.6 7.0 – 8.0			+4 to +35°C	
Step 2	Blocking of nonspecific binding sites		55 – 255		PBS/TBS	7.2 – 8.0		*e.g.,* 0.5 – 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 to +35°C	10 – 30 min
Step 3	Primary antibody	monoclonal/ polyclonal antibody	50 – 250	0.2 – 5	PBS/TBS	7.2 – 8.0	0.05-0.1% Tween 20	*e.g.,* 0.5 - 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+4 to + 35°C	1 – 18 h
Step 4	Anti-species (primary) antibody biotin-labeled	*e.g.,* anti-mouse Ig-biotin	50 – 250	0.05 – 2	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	*e.g.,* 0.5 - 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 to +35°C	1 – 4 h
Step 5	Streptavidin(SA)-enzyme or streptavidin plus biotin-(Bi)-enzyme	a) SA-alk. phosphatase b) SA-b-galactosidase c) SA-peroxidase a) SA+Bi-alk. phosphatase b) SA+Bi-b-galactosidase c) SA+Bi-peroxidase	50 – 250	dilution see recommended concentration	PBS/TBS	7.2 – 8.0	0.05-0.1% Tween 20	*e.g.,* 0.5 - 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 to +35°C	
Step 6	Substrate	a) pNPP b) oNPG/CPRG/MUG resorufin-gal c) ABTS/TMB/oPD	50 – 250	substrate specific	substrate buffer	sub-strate specific			+20 to +35°C	10 – 120 min
Step 7	Evaluation – visually – photometrically									
Between steps 3 – 6	Washing	wash buffer	250 – 300		PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20		+20 to +35°C	3 × 5 min

Tab. 2: Procedure for Immunoblotting

	Procedure/ Incubation	Reagent	Volume for 96 cm^2 ml	Reagent concentration µg/ml	Buffer	pH	Detergent	Protein addition	Incubation temperature	Incubation time
Step 1	Electrophoretic separation – SDS/native PAGE – 2-D-PAGE – agarose gel-electrophoresis – isoelectric focusing									
Step 2	Transfer to nitrocellulose membranes									
Step 3	Blocking of nonspecific binding sites		20		PBS/TBS	7.2 – 8.0		*e.g.,* 1% BSA/OVA/ gelatin (w/v)	+20 to +35°C	10 – 30 min
Step 4	Primary antibody	monoclonal/ polyclonal antibody to antigen	20	0.2 – 5	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	*e.g.,* 0.5 - 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+4 to +35°C	1 – 18 h
Step 5	Anti-species (primary) antibody biotin-labeled	*e.g.,* anti-mouse Ig-biotin	20	0.05 – 2	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	*e.g.,* 0.5 - 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 to +35°C	1 – 4 h
Step 6	Streptavidin(SA)-enzyme or streptavidin plus biotin-(Bi)-enzyme	a) SA-alk. phosphatase b) SA-b-galactosidase c) SA-peroxidase a) SA+Bi-alk. phosphatase b) SA+Bi-b-galactosidase c) SA+Bi-peroxidase	20	dilution see recommended concentration	PBS/TBS		0.05 – 0.1% Tween 20	*e.g.,* 0.5 - 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 to +35°C	0.5 – 2 h

	Procedure/ Incubation	Reagent	Volume for 96 cm^2 ml	Reagent concen-tration µg/ml	Buffer	pH	Detergent	Protein addition	Incubation tempera-ture	Incubation time
Step 7	Substrate	a) Fast Red/NABP, X-PO4/NBT b) X-gal, Fast Garnet/BNG c) DAB, AEC, chloro-naphthol	20	substrate specific	substrate buffer	substrate specific			+20 to +35°C	10 – 60 min
Step 8	Evaluation – visually – densito-metrically									
Between steps 3 – 6	Washing	wash buffer	20		PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20		+20 to +35°C	3 × 5 min

Tab. 3: Procedure for Immunohistochemistry or Immunocytochemistry

	Procedure/ Incubation	Reagent	Volume µl	Reagent concen-tration	Buffer	pH	Detergent	Protein addition	Incubation tempera-ture	Incubation time
Step 1	Preparation of – frozen section – paraffin embedded section – cell prepara-tions fixed on slides									
Step 2	Pretreatment, if necessary									
	– dewaxing	xylol	20 – 100						+18 to +25°C	10 min
	– treatment with proteases	trypsin/pepsin	20 – 100	0.1%	PBS	7.8			+18 to +25°C	30 min
Step 3	Blocking of nonspecific binding sites		20 – 100		PBS/TBS	7.2 – 8.0		*e.g.,* 1% BSA/OVA (w/v) serum undiluted	+20 to +35°C	10 – 30 min
Step 4	Primary antibody	monoclonal/ polyclonal antibody	20 – 100	2 – 40 mg/ml	PBS/TBS	7.2 – 8.0		*e.g.,* 1% BSA/OVA (w/v) 10% serum	+20 to +35°C	30 – 120 min
Step 5	Anti-Species (primary) antibody biotin-labeled	*e.g.,* anti-mouse Ig-biotin	20 – 100	0.1 – 5 mg/ml	PBS/TBS	7.2 – 8.0		*e.g.,* 1% BSA/OVA (w/v) 10% serum	+20 to +35°C	30 – 120 min
Step 6	Strepavidin(SA)-enzyme or streptavidin plus biotin-(Bi)-enzyme	a) SA-alk. phosphatase b) SA-b-galactosidase c) SA-peroxidase a) SA+Bi-alk. phosphatase b) SA+Bi-b-galactosidase c) SA+Bi-peroxidase	20 – 100	dilution see recom-mended concen-tration	PBS/TBS	7.2 – 8.0		*e.g.,* 1% BSA/OVA (w/v) 10% serum	+20 to +35°C	30 – 120 min
Step 7	Substrate	a) Fast Red/NABP, X-PO4/NBT b) X-gal, Fast Garnet/BNG c) DAB, AEC, chloro-naphthol	20 – 100	substrate specific	substrate buffer	substrate specific			+20 to +35°C	15 – 60 min
Step 8	Evaluation light microscope									
Between steps	Washing	wash buffer			PBS/TBS	7.2 – 8.0			+20 to +35°C	3 × 5 min

2. Supplementary Information

2.1 Conventions

Text Conventions

To make information consistent and easy-to-read, the following text conventions are used in this document:

Text Convention	Usage
Asterisk *	Denotes a product available from Roche Diagnostics.

2.2 Changes to Previous Version

- Editorial changes.

2.3 References

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany