For life science research only.
Not for use in diagnostic procedures.

Streptavidin Conjugates
from *E. coli* K12

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Cat. No. 11 089 161 001	Streptavidin-AP Conjugate 1,000 U
Cat. No. 11 089 153 001	Streptavidin-POD Conjugate 500 U

Store Streptavidin-AP Conjugate at +2 to +8°C.
Store Streptavidin-POD Conjugate at −15 to −25°C.

1. General Information

1.1. Contents

Label	Function / description	Catalog number	Content
Streptavidin-AP Conjugate	Solution, stabilized	11 089 161 001	1 vial, 1 ml (1,000 U)
Streptavidin-POD Conjugate	Solution, stabilized	11 089 153 001	1 vial, 500 U

1.2. Storage and Stability

Storage Conditions (Product)

When stored as shown in the table, the products are stable through the expiry date printed on the label.

Label	Storage
Streptavidin-AP Conjugate	Store at +2 to +8°C. ⚠ ***Do not freeze.***
Streptavidin-POD Conjugate	Store at −15 to −25°C. ⓘ *Once thawed, store at +2 to +8°C. Do not refreeze.*

1.3. Additional Equipment and Reagent required

For preparation of buffers and solutions

ⓘ *See section,* **Working Solution** *for information on preparing solutions.*

- Phosphate buffered saline* (PBS)
- Tris-buffered saline (TBS)
- $NaHCO_3/Na_2CO_3$

For inhibition of endogeneous alkaline phosphatase on tissue sections

- 1 mM Levamisol in PBS

Protein additives and blocking reagents

- Bovine serum albumin* (BSA)
- Ovalbumin (OVA)
- Gelatin, dried milk powder or animal sera

Detergents

- Tween 20* (v/v)
- Triton X-100* (v/v)

Preservatives

Sodium azide, thymol, or Bromo-nitrodioxane.

Substrate solutions for alkaline phosphatase

ⓘ *See section,* **Working Solution** *for information on additional reagents and preparing solutions.*

- pNPP
- Fast Red, TR salt/NABP
- Fast Red, TR salt/NAMP
- New Fuchsin/NABP
- X-phosphate/NBT*
- Tris-HCl*

Substrate solutions for Peroxidase

- ABTS*
- 3,3′,5,5′-Tetramethylbenzidine (TMB)
- 1,2-phenylenediamine (oPD)
- DAB Diaminobenzidine
- 3-Amino-9-ethyl carbazole (AEC)
- 4-Chloro-1-naphtol (CN)
- Tris-HCl*

1.4. Application

The 2 conjugates are used for the detection of biotin-labeled substances, such as biotinylated antibodies in several immunological detection systems:

- ELISA
- Immunohistochemistry and immunocytochemistry
- Immunoblotting
- Screening of monoclonal antibodies

Biotinylated products, such as antibodies or lectins can be purchased or synthesized. Biotin can be easily conjugated to several substances, for example, amino, carboxyl, aldehyde, and thiol groups, and nucleic acids.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Frozen sections

Ideal frozen sections (4 to 5 µm) are obtained from shock-frozen tissue samples.

1. Air dry the frozen sections and fix them with acetone for 10 minutes at −15 to −25°C.
2. Remove excess acetone by drying or by washing with PBS.

Paraffin sections

1. Dewax formalin-fixed, paraffin-embedded tissue sections by extracting them twice with xylene, 10 minutes per extraction.
2. Wash the xylene out with steadily decreasing concentrations of ethanol, down to 70%.
3. Wash the sections in double-distilled water and equilibrate in PBS.
4. Depending on the type of primary antibody, treat with proteases, such as 0.1% Trypsin* (w/v), or Pepsin*.

Cell suspensions

1. Adjust cells to 10^6 to 10^7/ml in medium and add the primary antibody to the untreated sample.
2. If interfering substances are present in the medium, centrifuge the cell suspension for 20 minutes at +15 to +25°C at 400 to 600 × *g*, wash with PBS, and take the cells up in the desired medium, for example, 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

1. Smear one drop of the sample, such as blood with a cover glass slide, let air-dry and fix with acetone, methanol or methanol/acetic acid (95%/5% [v/v]) for 10 minutes at −15 to −25°C.

2. Wash out the fixative with PBS or let dry.

Cytospin preparations

1. Centrifuge cell suspensions at approximately 100 × *g* for 10 minutes at +15 to +25°C onto a glass slide using a Cytospin apparatus.

2. Remove apparatus, suck off excess liquid, wash the cells for a short time in PBS, and fix them in acetone or methanol.

3. Wash the fixative off with PBS or let dry.

Coverslip preparations

1. Use forceps to pick up coverslips that contain cells, then remove excess liquid carefully by suction.

2. Wash the cells briefly in PBS and fix the cells in acetone or methanol for 10 minutes at −15 to −25°C.

3. Wash the fixative off with PBS or let dry.

Tissue culture chamber/slide

1. Carefully remove liquid by suction and wash cells briefly in PBS.

2. Fix the cells in acetone or methanol for 10 minutes at −15 to −25°C.

3. Wash out the fixative with PBS or let dry.

General Considerations

Alkaline phosphatase

Alkaline phosphatase is a widely used marker enzyme for immunoassays. Stable, water insoluble products are formed using the sensitive substrate system with X-phosphate/NBT.

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

- A unit refers to the enzyme unit of alkaline phosphatase, or POD.
- Biotin, also known as vitamin H, is a readily water-soluble substance with a molecular weight of 244 Da. It binds with one of the highest naturally known affinities of 10^{-15} mol^{-1} to avidin, a glycoprotein (M_r 66 kDa, pI 10) from egg white and to streptavidin. Streptavidin is a non-glycosylated protein (M_r 60 kDa, pI 7.2/7.4) from the bacterium *Streptomyces avidinii* and comprises four binding sites for biotin. The binding is almost irreversible. For comparison, antibodies bind to their antigens in a range between 10^{-7} to 10^{-11} mol^{-1}.
- Both proteins can be conjugated with numerous signal providing substrates, such as gold, enzymes, and fluorescent dyes so that the most favorable system can be selected. A further variation consists of labeling the signal-providing enzymes with biotin and then detecting the biotin conjugate either as a complex of streptavidin plus biotin-enzyme, or by initial incubation with streptavidin and subsequent addition of the biotinylated enzyme.
- An isoelectric point in the neutral range and the absence of carbohydrate fractions makes 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.

Assay requirements for ELISA, immunoblotting, immunohistochemistry

The following information is intended as a guideline to establish an immunotest. The performance of the system can be adjusted to individual assay requirements by variation of parameters, such as the quality of the reagents, concentrations, buffers, incubation times, and temperature.

Reagents

Use only analytical quality reagents.

Solid phases for ELISA and immunoblotting

Microplates

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 types of membranes for immunoblotting.

- The most frequently used for adsorption is nitrocellulose, but nylon- and PVDF (polyvinylidene difluoride) membranes can also be used.
- For covalent binding, use activated cellulose paper.
- For ionic binding, use DEAE (diethylaminoethyl) paper.

Fixation on Terasaki plates

1. Precoat the wells with 10 µl Poly-L-Lysine* in PBS, 25 µg/ml for 20 minutes at +15 to +25°C and wash the wells twice in PBS.

2. Pipette 10^4 to 10^6/ml cells suspended in PBS into the wells and centrifuge at 100 to 200 × *g* for 15 minutes at +15 to +25°C.

3. Remove the supernatant by suction, add 0.01% glutardialdehyde solution (v/v), and incubate for 5 minutes at +15 to +25°C.

4. Wash the wells once with PBS and block the binding sites with 1% bovine serum albumin (w/v) in PBS, for 20 minutes at +15 to +25°C.

Paraformaldehyde fixation

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

1. Prepare 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 in an ice-bath.

2. Wash tissue sections or cells, respectively, on the slide twice with PBS and fix for 15 minutes at 0 to +2°C in an ice-bath in paraformaldehyde solution.

3. Wash the preparation once with PBS and subsequently incubate with 0.1% Triton* X-100 (v/v), in PBS at +15 to +25°C.

   Incubate 1 to 5 seconds for surface antigens, 5 minutes for permeation of the cell membrane.

4. Wash the preparation three times with PBS and block nonspecific binding sites with fetal calf serum.

Protein additive/blocking reagents

Bovine serum albumin* (BSA), ovalbumin (OVA), gelatin, dried milk powder, or animal sera corresponding to the secondary antibody are frequently used. Concentrations are between 0.2 to 2% (w/v). Sera are used at a ratio of 10% (v/v).

Detergents

- 0.02 to 0.1% Tween 20* (v/v)
- 0.02 to 0.1% Triton X-100* (v/v)

Detergents are frequently used in ELISA. For histochemistry, detergents are generally omitted and their use in immunoblotting must be evaluated.

Preservatives

For best results, add preservatives to the buffers and solutions in the order indicated to prevent microbial contamination. Sodium azide at 0.1% (w/v) is frequently used to inactivate peroxidase; thymol (up to saturation point) is also often used. An alternative to thymol is 5-Bromo-5-nitro-1.3-dioxane.

Substrate solutions

The substrates used depend on the application, for example, substrates with water-soluble products for ELISA or substrates with water-insoluble products for immunohistochemistry and immunoblotting.

For best results, solutions should be prepared just before use.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

For Buffers and Solutions	
Solution	**Preparation/Composition**
Phosphate buffered saline (PBS)*	10 to 20 mM potassium phosphate or sodium phosphate or potassium-sodium phosphate, pH 7.2 to 7.4, 150 mM sodium chloride.
Tris-buffered saline (TBS)	20 to 100 mM Tris-HCl*, pH 7.5 to 8.0, 150 mM sodium chloride.
$NaHCO_3/Na_2CO_3$	50 mM sodium hydrogen carbonate/sodium carbonate, pH 9.6.
For Inhibition of Endogenous Enzymes on Tissue Sections	
For Alkaline Phosphatase	1 mM Levamisol in PBS.
For Peroxidase	▪ 0.074% HCl (v/v) in ethanol, A.R. (fixation step). ▪ 0.3 to 3% H_2O_2 (v/v) in PBS, incubate for 30 minutes at +15 to +25°C. ▪ 0.1% phenylhydrazine (w/v) in PBS, incubate for 1 hour at +37°C with freshly prepared solution.
For Alkaline Phosphatase ⚠ ***Prepare all substrate solution fresh.***	
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.

For Peroxidase ⚠ ***Prepare all substrate solution fresh.***	
ABTS* (2,2′-Azino-di-(3-ethylbenzthiazoline sulfonate)	100 mg ABTS substrate in 3.25 mM sodium perborate, 39.8 mM citric acid, 60 mM disodium hydrogen phosphate, pH 4.4 to 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 dimethylsulfoxide. ▪ 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.

2.2. Protocols

ELISA protocol

The following table is an example of a hybridoma screening.

Step	Protocol/ incubation	Reagent	Volume [µl]	Reagent conc. [µg/ml]	Buffer	pH	Detergent	Protein addition	Incubation temperature	Incubation time
1	Coating of microplates	Antigen	50 – 250	0.2 – 5	$NaHCO_3$/ Na_2CO_3	9.6	–	–	+4 – +35°C	–
					PBS/TBS	7.0 – 8.0				
2	Blocking of nonspecific binding sites	–	55 – 255	–	PBS/TBS	7.2 – 8.0	–	For example, 0.5 – 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 – +35°C	10 – 30 min
3	Primary antibody	Monoclonal/ polyclonal antibody	50 – 250	0.2 – 5	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	For example, 0.5 – 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+4 – +35°C	1 – 18 h
4	Anti-species (primary) antibody biotin-labeled	For example, anti mouse Ig-biotin	50 – 250	0.05 – 2	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	For example, 0.5 – 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 – +35°C	1 – 4 h

5	Streptavidin-enzyme, or	Streptavidin - AP	50 – 250	Dilution, see section, **Working Concentration**.	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	For example, 0.5 – 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 – +35°C	–
		Streptavidin - POD								
	Streptavidin plus biotin-(Bi)-enzyme	Streptavidin + Bi - AP								
		Streptavidin + Bi - POD								
6	Substrate	pNPP	50 – 250	Substrate specific	Substrate buffer	Substrate specific	–	–	+20 – +35°C	10 – 120 min
		ABTS, TMB, oPD								
7	Evaluation visually or photometrically									
Between Steps 3 to 6	Washing	Wash buffer	250 – 300	–	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	–	+20 – +35°C	3 × 5 min

Immunoblotting protocol

Step	Protocol/ incubation	Reagent	Volume [µl]	Reagent conc. [µg/ml]	Buffer	pH	Detergent	Protein addition	Incubation temperature	Incubation time
1	Electrophoretic separation ▪ SDS/native PAGE ▪ 2-D-Page ▪ Agarose gel electrophoresis ▪ Isoelectric focusing									
2	Transfer to nitrocellulose membranes									
3	Blocking of nonspecific binding sites	–	20	–	PBS/TBS	7.2 – 8.0	–	For example, 1% BSA/ OVA/gelatin (w/v)	+20 – +35°C	10 – 30 min
4	Primary antibody	Monoclonal/ polyclonal antibody to antigen	20	0.2 – 5	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	For example, 0.5 – 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+4 – +35°C	1 – 18 h
5	Anti-species (primary) antibody biotin-labeled	For example, anti mouse Ig-biotin	20	0.05 – 2	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	For example, 0.5 – 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 – +35°C	1 – 4 h
6	Streptavidin-enzyme, or	Streptavidin - AP	20	Dilution, see section, **Working Concentration**.	PBS/TBS	–	0.05 – 0.1% Tween 20	For example, 0.5 – 2% BSA/OVA (w/v) 0.2 – 1% gelatin (w/v)	+20 – +35°C	0.5 – 2 h
		Streptavidin - POD								
	Streptavidin plus biotin-(Bi)-enzyme	Streptavidin + Bi - AP								
		Streptavidin + Bi - POD								
7	Substrate	Fast Red/ NABP, X-PO_4/ NBT	20	Substrate specific	Substrate buffer	Substrate specific	–	–	+20 – +35°C	10 – 60 min
		DAB, AEC, chloro-naphthol								
8	Evaluation visually or densitometrically									
Between Steps 3 to 6	Washing	Wash buffer	20	–	PBS/TBS	7.2 – 8.0	0.05 – 0.1% Tween 20	–	+20 – +35°C	3 × 5 min

Immunohistochemistry or immunocytochemistry protocol

Step	Protocol/ incubation	Reagent	Volume [µl]	Reagent conc. [µg/ml]	Buffer	pH	Detergent	Protein addition	Incubation temperature	Incubation time
1	Preparation of: ▪ Frozen sections ▪ Paraffin-embedded sections ▪ Cell preparations fixed on slides									
2	Pretreatment, if necessary: Dewaxing	Xylol	20 – 100	–	–	–	–	–	+18 – +25°C	10 min
	Pretreatment, if necessary: With proteases:	Trypsin/ pepsin	20 – 100	0.1%	PBS	7.8	–	–	+18 – +25°C	30 min
3	Blocking of nonspecific binding sites	–	20 – 100	–	PBS/TBS	7.2 – 8.0	–	For example, 1% BSA/ OVA (w/v) Serum undiluted	+20 – +35°C	10 – 30 min
4	Primary antibody	Monoclonal/ polyclonal antibody	20 – 100	2 – 40 mg/ml	PBS/TBS	7.2 – 8.0	–	For example, 1% BSA/ OVA (w/v) 10% serum	+20 – +35°C	30 – 120 min
5	Anti-species (primary) antibody biotin-labeled	For example, anti mouse Ig-biotin	20 – 100	0.1 – 5 mg/ml	PBS/TBS	7.2 – 8.0	–	For example, 1% BSA/ OVA (w/v) 10% serum	+20 – +35°C	30 – 120 min
6	Streptavidin-enzyme, or	Streptavidin - AP	20 – 100	Dilution, see section, **Working Concentration**.	PBS/TBS	7.2 – 8.0	–	For example, 1% BSA/ OVA (w/v) 10% serum	+20 – +35°C	30 – 120 min
		Streptavidin - POD								
	Streptavidin plus biotin-(Bi)-enzyme	Streptavidin + Bi - AP								
		Streptavidin + Bi - POD								
7	Substrate	Fast Red/ NABP, X-PO_4/ NBT	20 – 100	Substrate specific	Substrate buffer	Substrate specific	–	–	+20 – +35°C	15 – 60 min
		DAB, AEC, chloro-naphthol								
8	Evaluation by light microscopy									
Between Steps 3 to 6	Washing	Wash buffer	–	–	PBS/TBS	7.2 – 8.0	–	–	+20 – +35°C	3 × 5 min

2.3. Parameters

Working Concentration

Conjugate	Application	Starting Concentration [mU/ml]	Dilution of Stock Solution	Number of Reactions
Streptavidin-AP Conjugate	ELISA	50 – 1,000	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-POD Conjugate	ELISA	5 – 500	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

Working concentration of conjugate depends on application and substrate. The following concentrations should be taken as a guideline:
- ELISA: 50 to 200 mU/ml
- Immunohistocytochemistry: 250 to 1,000 mU/ml
- Western blot: 200 to 500 mU/ml

3. Additional Information on this Product

3.1. Test Principle

Preparation

Streptavidin-AP Conjugate

① Streptavidin from *E. coli* K12 was coupled to alkaline phosphatase (AP) from calf intestine via heterobifunctional linkers.

② The conjugate was then purified using column chromatography.

③ 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).

Streptavidin-POD Conjugate

① Streptavidin from *E. coli* K12 was coupled to β-peroxidase (POD) from horseradish using the periodate-method.

② The conjugate was then purified using column chromatography.

③ 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).

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Editorial changes.
Formulation of Streptavidin-POD Conjugate (11089153001) is changed from Lyophilized to Solution.
Chapter 1.2 Storage and Stability, storage conditions of Streptavidin-POD Conjugate updated.
Chapter 1.2 Storage and Stability, subchapter regarding reconstitution of Streptavidin-POD Conjugate is no longer relevant and has been removed.
References to the Streptavidin-β-Gal Conjugate removed due to outphase of product.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Tris hydrochloride	500 g	10 812 846 001
BCIP	250 mg	10 760 994 001
	1 g	11 585 002 001
NBT	5 g	11 585 029 001
ABTS	2 g	10 102 946 001
Bovine Serum Albumin Fraction V	50 g	10 735 078 001
	100 g, *Not available in US*	10 735 086 001
	500 g, *Not available in US*	10 735 094 001
	1 kg, *Not available in US*	10 735 108 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001

4.4. Trademarks

ABTS is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany