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

StreptaWell

Version: 11
Content Version: November 2021

Cat. No. 11 734 776 001	StreptaWell, 96-wells, transparent, C-bottom 15 plates
Cat. No. 11 664 778 001	StreptaWell, 12 × 8-well strips and frame, transparent, C-bottom 5 plates
Cat. No. 11 645 692 001	StreptaWell High Bind, 12 × 8-well strips and frame, transparent, C-bottom 5 plates
Cat. No. 11 989 685 001	StreptaWell High Bind, 96-wells, transparent, C-bottom 15 plates

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Label	Function / description	Catalog number	Content
StreptaWell, High Bind (transparent, 96-wells)	Ready-to-use, C-bottom plates. *No extra rehydration steps required.*	11 989 685 001	15 plates
StreptaWell, (transparent, 96-wells)		11 734 776 001	15 plates
StreptaWell, High Bind (transparent, 12 × 8-well strips)		11 645 692 001	5 plates
StreptaWell, (transparent, 12 × 8-well strips)		11 664 778 001	5 plates

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Label	Storage
StreptaWell, High Bind (transparent, 96-wells)	Store dry at +2 to +8°C. ⚠ ***Keep protected from light.***
StreptaWell, (transparent, 96-wells)	
StreptaWell, High Bind (transparent, 12 × 8-well strips)	
StreptaWell, (transparent, 12 × 8-well strips)	

1.3. Additional Equipment and Reagent required

For labeling of molecules

- Biotin Protein Labeling Kit*
- DIG DNA Labeling Kit*

For ELISA

- PBS* or TBS
- BSA*
- Tween 20* (optional)
- NaCl (optional)
- EDTA (optional)

Substrates for detection

- CPRG*
- ABTS*
- BM Blue POD Substrate, soluble*

1.4. Application

StreptaWell plates can be used for colorimetric and fluorescent protein immunoassays.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Streptavidin-coated microplates are available in two binding capacities.
The different binding capacities result from different coating procedures using different starting materials.
- StreptaWell (regular-binding capacity), and
- StreptaWell, High Bind (high-binding capacity)

Coating specifications

Specification	StreptaWell 96	StreptaWell 96 High Bind
SA-coated area (indicated as volume)	≥300 µl	≥300 µl
Blocked volume	≥320 µl	≥320 µl
Total biotin binding capacity (competition assay)[1]	≥5 ng/well ≥20 pmol/well ≥70 nM	≥20 ng/well ≥80 pmol/well ≥200 nM
Total binding capacity (for biotin-labeled antibodies)	1.5 µg/well	1.5 µg/well
Coating variance (between individual wells)	<5%	<5%
CV (between different plates)	<10%	<10%
SA-leaching	<5 ng/well	<5 ng/well

[1] Well: 300 µl

Detection methods

StreptaWell plates can be used with the following detection methods.

StreptaWell	Colorimetric	Chemiluminescent	Fluorescent
Transparent	++	-	+

Homogeneity and production variances

The proprietary coating process guaranties highly homogeneous and reproducible coatings:
- Minimal variance between individual wells (intra-assay variance).
- Minimal variance between different plates (inter-assay variance).
- High lot-to-lot reproducibility.
- High signal-to-noise ratio.
- Low background.
- Almost no leaching.

⚠ ***Do not sonicate microplates or wells.***

Substrates for detection

StreptaWell	Colorimetric	Chemiluminescent	Fluorescent
Transparent	CPRG*, ABTS*, BM Blue POD*	-	+

2.2. Protocols

Labeling of proteins, peptides, and small molecules

Labeling of proteins, peptides, and small molecules, such as haptens can be performed under mild conditions via free amino groups, sulfhydryl groups, disulfide bridges, or oxidized sugar residues (aldehyde or keto groups). In general, label the proteins via free amino groups (lysyl residues).

- Labeling with biotin normally has no effect on the properties of proteins.
- For small molecules, such as haptens and oligopeptides, the label must be conjugated to a part of the molecule not essential for function.
- Often a spacer between label and hapten or ligand is advantageous to allow optimal interaction with antibodies or receptors.

Labeling of nucleic acids

Perform nucleic acid labeling chemically or enzymatically with reagents or kits. In molecular biology, double labeling with biotin and digoxigenin is often used to analyze nucleic acids in polymerization and hybridization experiments.

ELISA protocol

When possible, allow biotinylated components to bind to streptavidin under physiological buffer conditions.

- Immobilization of biomolecules via streptavidin/biotin interaction is at least as effective as direct coating to physically activated surfaces. For small molecules, oligonucleotides, or peptides, the binding of biotinylated components will be much more efficient.
- After washing out excess biotinylated substance, all additional steps may follow the standard protocol as optimized for a particular parameter.

The following table shows the components needed for the ELISA.

Step	Composition/ Preparation	Volume [µl]	Time [Min]	Temperature [°C]
Binding of biotinylated component.	PBS* or TBS containing 0.1% BSA*.	50 – 100	15 – 60	+15 to +25 or +35
Washing	PBS* or TBS containing 0.1% BSA* and/or additional additives.	300 (each wash cycle)	3 – 5 times with 5 minutes incubation between individual washes.	+15 to +25
Incubation of antibodies, antigen, etc.	PBS* or TBS containing 0.1% BSA* and/or additional additives, depending on the components used.	100 – 150	60	+15 to +25 or +35
Secondary detection component.		200		
Colorimetric, chemiluminescence, or fluorescence substrate solutions.	Prepare solutions according to the manufacturer's protocol or use ready-to-use reagents.	250 (including volume of trigger solutions, if required).	Depends on enzyme/substrate system.	+15 to +25

Antibody-conjugate concentration

When changing to fluorescent or chemiluminescent detection, the concentrations of the conjugates often have to be adapted. Initially, the conjugate concentration should be used as recommended by the supplier or as optimized for a particular colorimetric assay. If the conjugate contributes to nonspecific binding, its concentration may be lowered down to 1:10.

Reduction of nonspecific binding

Optimizing nonspecific binding might be a prerequisite for highly sensitive detection. To reduce background, additional components may be added to washing, incubation, and conjugate buffers, and/or the concentrations of the specific interacting components may be lowered. The following additives may be used at the designated concentrations.

Additive	Concentration
Salt (NaCl)	0.5 – 1.0 M
Complexing agent (EDTA)	1 – 5 mM
Detergent (Tween 20*)	0.05 – 0.1%
Protein (BSA*, serum, casein, milk powder)	0.1 – 1%

Washing conditions

Most interactions which contribute to nonspecific binding are of low/intermediate affinity and therefore reversible in character. Prolonged intervals between individual washes (at least 3 repeated washes) favor dissociation from nonspecific binding sites.

Handling concentrated samples

Dilute samples exceeding the measuring range with incubation buffer and repeat the ELISA. This dilution factor should be taken into account when calculating content.

2.3. Parameters

Affinity/Binding Capacity

The biotin binding capacity, see section, **General Considerations** is identical to the number of biotin binding sites present. Depending on the size and sterical properties of a given biomolecule, the actual molar binding capacity may often be below this maximum value.

Factors influencing binding capacity

Even though the binding capacity could be reduced to some extent, the integrity of the coating and the stability of the streptavidin-biotin interaction has proven to be remarkably resistant to a variety of harsh conditions:

- Common molecular biology buffers, such as SSC, TEN, RIPA, TBS, etc.
- 4 M guanidinium thiocyanate: +15 to +25°C, 1 hour
- 4 M Urea: +37°C, 1 hour
- 50% formamide: +56°C, 1 hour
- 1% SDS: +37°C, 1 hour

  Some detergents may influence the properties of the StreptaWell plate in concentrations >0.1 to 1%.
- pH 4 to 10
- Elevated temperatures up to +75°C.

  Do not heat above +75°C.

Sensitivity

Potential sensitivity-limiting factors for a microplate assay include:

- Affinity of the specifically interacting components.
- Sensitivity of the detection system.
- Nonspecific binding (signal-to-noise).

Maximum sensitivity of the detection method can only be achieved if the affinities of the interacting components are not limiting for the assay, and the total nonspecific signal is well below 0.1% of the total signal.

3. Troubleshooting

Observation	Possible cause	Recommendation
Week or no signal present.	Incorrect instrument settings.	Check instrument settings.
	Inactive, expired enzymes.	Check activity of marker enzyme/ molecule.
	Ingredients in water that influence the test negatively.	Always use double-distilled water for reconstitution and preparing the working solutions. ⚠ ***Make sure that the water is not contaminated with microbes.***
	Interference of buffer components with substrate.	Check conjugate buffer for incompatible components, such as NaN_3, SH-reagents.
	Inadequate incubation time and temperature.	Check protocol, such as the incubation times, temperatures, buffer conditions, etc., and concentrations of primary antibody or antigen.
	Substrate or vial used to aliquot substrate contaminated.	Check substrate reagent for storage conditions and biological contamination. ⚠ ***Use freshly prepared reagent.***
		Check integrity of positive control.
High background signal.	Washing procedure not efficient.	Prolong washing procedure, such as number of washes and interval between washes.
	Nonspecific interaction of buffer additives.	Try different additives with the washing and incubation buffers to block nonspecific interactions.
	Inadequate concentration of detection component, such as antibodies.	Modulate concentrations for detection components, for example, the primary/ secondary antibody.

4. Additional Information on this Product

4.1. Test Principle

Streptavidin-based assay system

The modular streptavidin-based assay system is composed of more than 100 different items which enable the setup of almost any biochemical assay. The system consists of universal modules and parameter-specific components, see Figure 1.

- The universal modules are identical for most applications, comprised of a streptavidin-coated microplate (StreptaWell plate), anti-digoxigenin-enzyme conjugate, and a set of different substrate alternatives to generate a chemiluminescent, fluorescent, or colorimetric signal.
- The parameter-specific components which have to be designed according to the parameter of interest are biotin-labeled for immobilization purposes, and digoxigenin (DIG)-labeled for detection and quantification. The techniques associated with labeling the parameter-specific components, such as peptides, proteins, and nucleic acids are well established.

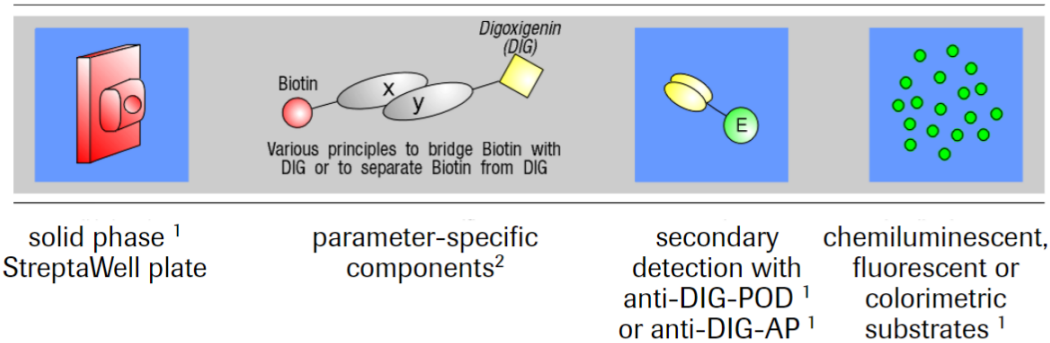

Fig. 1: Principle of the Modular Streptavidin-based Screening System.

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
BM Blue POD Substrate, soluble	100 ml	11 484 281 001
CPRG	250 mg	10 884 308 001
ABTS Solution	3 x 100 ml, for 1,500 to 3,000 reactions	11 684 302 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
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
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Biotin Protein Labeling Kit	1 kit, 5 labeling reactions	11 418 165 001
DIG DNA Labeling Kit	1 kit, 40 labeling reactions of 10 ng to 3 µg DNA	11 175 033 910

5.4. Trademarks

MODULAR, ABTS and STREPTAWELL are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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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