Thermo SCIENTIFIC

PRODUCT INFORMATION

Biotin Chromogenic Detection Kit

#K0661, #K0662

www.thermoscientific.com/onebio

#K___
Lot __
Expiry Date __

CERTIFICATE OF ANALYSIS

The kit has been tested in dot-blot hybridization on the Nylone membrane Hybond™-N+ Nylon Membrane (Amersham, #RPN119B). The biotin-labeled probe was generated by a random primer labeling reaction with the Thermo Scientific Biotin DecaLabel DNA Labeling Kit (#K0651). Hybridization and detection procedures were performed according to the Hybridization procedure (p.6) and the Detection Protocol (p.5).
The labeled-probe spot corresponding to 30 fg of the target DNA was easily detected after overnight colour development.

Quality authorized by:  Jurgita Zilinskiene

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COMPONENTS OF THE KIT

Component	#K0661 **for 10 rxns**	#K0662 **for 30 rxns**
Streptavidin-AP Conjugate	40 µL	120 µL
50X BCIP/NBT Solution	4 mL (2 × 2 mL)	12 mL (6 × 2 mL)
Blocking Reagent (powder)	5 g	15 g
10X Blocking/Washing Buffer	200 mL	600 mL (3 × 200 mL)
10X Detection Buffer	30 mL	90 mL

Materials and equipment to be supplied by the user

- Hybridization containers or bags for incubation/washing steps;
- Platform shaker for incubation/washing steps;
- High quality water (Milli-Q or double deionized);
- Labware for preparation and storage of solutions used in the experiments.

STORAGE

All components of the kit are stable up to the kit expiry date if stored correctly.

Component	**Storage conditions**
Streptavidin-AP Conjugate	-20°C
50X BCIP/NBT Solution	-20°C (protect from light)
Blocking Reagent (powder)	-20°C or 4°C
10X Blocking/Washing Buffer	-20°C or 4°C
10X Detection Buffer	4°C

DESCRIPTION

The Biotin Chromogenic Detection Kit is a convenient tool for the chromogenic detection of biotinylated nucleic acid probes. The kit is optimized to reproducibly provide high detection sensitivity with low background in applications such as Southern, Northern, dot and slot blotting, as well as screening of viral plaques and bacterial colonies. The detection limit of a target sequence is 30-100 fg.

PRINCIPLE

Biotin-labeled probe-target hybrids are detected with alkaline phosphatase-conjugated streptavidin (*see* Fig.1).

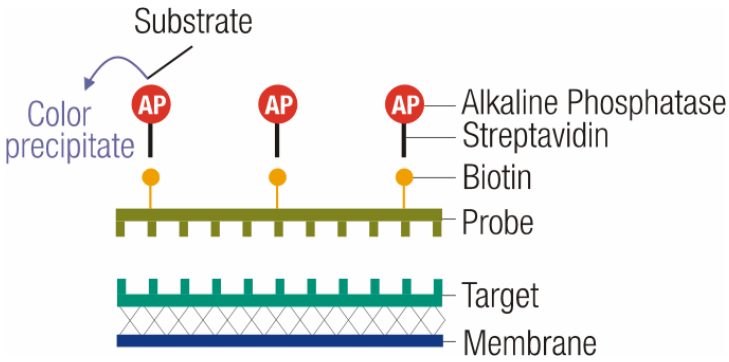

Fig. 1. Chromogenic detection of a biotin-labeled probe-target hybrid.

Streptavidin is a biotin-binding tetrameric protein from Streptomyces avidinii with a molecular weight of approximately 60 kDa. The streptavidin-biotin bond is the strongest known noncovalent biological interaction ($K_d \approx 10^{-15}$ M). Formation of such a complex is a very rapid process and, once formed, the complex is unaffected by external factors. Unlike avidin, streptavidin does not undergo any post-translational glycosylation. Its isoelectric point (pI 5-6) is ideal for use in the majority of applications. These properties result in high detection sensitivity and very low background.

Streptavidin is conjugated to alkaline phosphatase (AP), which facilitates chromogenic detection. Alkaline phosphatase cleaves the substrate, BCIP-T (5-bromo-4-chloro-3-indolyl phosphate, p-toluidine salt) which is supplemented with the enhancer chromogen NBT (nitro blue tetrazolium). This results in the formation of an insoluble blue precipitate, which appears as a well-defined spot or band at the reaction site on the membrane. No special equipment is required for chromogenic assays employing AP-conjugated streptavidin.

DETECTION PROTOCOL

The protocol is optimized for the detection of biotin-labeled probes hybridized to target nucleic acids on positively-charged membranes. It is not recommended for *in situ* hybridization techniques.

Preparation of assay solutions

All assay solutions should be prepared with high quality water (Milli-Q or double deionized).

Volumes of assay solutions required to develop a 100 cm^2 membrane in an appropriately-sized container are given below.

Assay Solution	Required Volume
Blocking/Washing Buffer	200 mL
Blocking Solution	50 mL
Diluted Streptavidin-AP Conjugate	20 mL
Detection Buffer	30 mL
Substrate Solution	10 mL

Scale the volumes of solutions according to the size of your membrane and container. Prepare sufficient solutions to allow the membrane to float freely in the tray.

Blocking/Washing Buffer. Dilute 1 volume of the concentrated 10X Blocking/Washing Buffer with 9 volumes of water. Diluted buffer can be stored at 4°C for 1 week. Add 0.1% sodium azide for a longer storage.

Blocking Solution. The Blocking Solution is prepared as a 1% (w/v) solution of Blocking Reagent in 1X Blocking/Washing Buffer. Weigh out the necessary amount of the Blocking Reagent, add the required volume of the Blocking/Washing Buffer, and stir the suspension on a magnetic stirrer until the Blocking Reagent completely dissolves.

Note

- Shaking of the suspension at 50-60°C facilitates dissolution of the Blocking Reagent.
- Once prepared, the Blocking Solution should be store in aliquots at -20°C.

Dilution of the Streptavidin-AP Conjugate. Dilute the concentrated Streptavidin-AP conjugate 5000-fold in Blocking Solution. Prepare sufficient solution to allow the membrane to float freely in a tray. The diluted solution should be prepared **just prior to use**.

Detection Buffer. Dilute 1 volume of the concentrated 10X Detection Buffer with 9 volumes of water. The diluted buffer can be stored at 4°C for 1 week. Add 0.1% sodium azide for a longer storage.

Substrate Solution. Dilute 1 volume of 50X BCIP/NBT Solution with 49 volumes of 1X Detection Buffer. Do not add 50X BCIP/NBT directly to the concentrated 10X Detection Buffer. The Substrate Solution should be prepared fresh **just prior to use.**

Detection procedure

Note. During all steps of the detection procedure, the membrane must float freely in the container and be evenly covered with solution. Solution volumes provided below have been calculated for a 100cm^2 membrane in an appropriately-sized container.

Step	Procedure
1	**Wash** the membrane (after hybridization/washing steps) in 30 mL of Blocking/Washing Buffer for 5 minutes at room temperature on a platform shaker with moderate shaking.
2	**Block** the membrane in 30 mL of the Blocking Solution for 30 minutes at room temperature with moderate shaking.
3	**Prepare** 20 mL of diluted Streptavidin-AP conjugate.
4	**Incubate** the membrane in 20 mL of diluted Streptavidin-AP conjugate for 30 minutes at room temperature with moderate shaking.
5	**Wash** the membrane at room temperature with moderate shaking as indicated below: a) Incubate with 60 mL of Blocking/Washing Buffer for 15 minutes. Discard the solution and repeat once with fresh Blocking/Washing Buffer. Discard the solution b) Incubate with 20 mL of Detection Buffer for 10 minutes and discard the solution.
6	**Perform the enzymatic reaction**. Incubate the membrane in 10 mL of freshly prepared Substrate Solution at room temperature in the dark. The blue-purple precipitate becomes visible after 15-30 minutes of incubation. For the highest sensitivity, allow the color to develop overnight.
7	**Stop the reaction**. Discard the substrate solution and rinse the membrane with water (Milli-Q or double deionized) for few seconds.
8	Discard the water and air-dry the developed membrane to document the results. **Note.** The membrane should not be dried if stripping and re-hybridization are planned in subsequent experiments.

SUPPLEMENTARY PROTOCOLS

Hybridization protocol

Solutions required:

100X Denhardt's solution
2%(w/v) BSA, 2%(w/v) Ficoll™, 2%(w/v) PVP (polyvinylpyrrolidone)

20X SSC (pH 7.0)
3 M NaCl
0.3 M Na_3 Citrate

Note. This hybridization procedure has been optimized for nylon membranes. The detection sensitivity may vary depending on the membranes supplied by different vendors.

Procedure

Step	Procedure
1	Prepare the following pre-hybridization solution (final concentration): 6X SSC 5X Denhardt's solution 0.5% SDS 50% (v/v) deionized formamide
2	Denature a 0.5 mg/mL aqueous solution of sonicated herring or salmon sperm DNA at 100°C for 5 minutes, and then chill on ice. Add the denatured DNA to the pre-hybridization solution to obtain a final DNA concentration of 50 µg/mL.
3	Place the membrane in an appropriately-sized container (plastic bag or hybridization bottle), add the pre-hybridization solution with denatured DNA (0.2 mL/cm^2) and pre-hybridize at 42°C for 2-4 hours with shaking.
4	Prepare the hybridization solution: a) Denature the biotin-labeled probe at 100°C for 5 minutes and chill on ice. b) Add the denatured probe to the pre-hybridization solution to obtain a final probe concentration of 25-100 ng/mL.
5	Discard the pre-hybridization solution and add the hybridization solution to the membrane (60 µL/cm^2).
6	Incubate overnight at 42°C with shaking.
7	Wash the membrane twice with 2X SSC, 0.1% SDS. Perform each wash for 10 minutes at room temperature.
8	Wash the membrane twice with 0.1X SSC, 0.1% SDS. Perform each wash for 20 minutes at 65ºC.
9	Remove excess liquid from the membrane by briefly placing it on filter paper.
10	Detect the biotin-labeled DNA as described above (*see* Detection Protocol).

Evaluation of labeling efficiency

The labeling efficiency is defined as the lowest detectable concentration of the labeled probe.

Step	Procedure
1	Prepare several dilutions of the biotin-labeled probe ranging from 1 ng/µL to 10 fg/µL, and then spot 1µL of each dilution onto a nylon membrane strip.
2	Air-dry the spotted probe at room temperature for 30-45 minutes. Alternatively the spots can be dried at 80°C for 10 minutes.
3	Place the membrane on a UV trans-illuminator (spotted side down) and cross link the probe to the membrane for 1-5 minutes. **Note.** The spotted membrane can be stored indefinitely at 4°C or at room temperature in a plastic bag until needed.
4	Place the membrane strip into an appropriately-sized hybridization bag and perform the detection procedure as described above (*see* Detection Procedure). Adjust the volumes of the solutions to the size of the membrane strip and the bag.

Use the following guidelines for calculation of the necessary solution volumes:

Blocking Solution	300 µL/cm^2 of the membrane
Diluted Streptavidin-AP Conjugate	200 µL/cm^2 of the membrane
Blocking/Washing Buffer	600 µL/cm^2 of the membrane
Detection Buffer	200 µL/cm^2 of the membrane
Substrate Solution	100 µL/cm^2 of the membrane

The labeling efficiency is acceptable if the **30-100 fg** spot of the labeled probe is easily detected after overnight incubation.

TROUBLESHOOTING

Problem	Possible cause and solution
Low signal (sensitivity)	**The probe concentration is too low.** Increase the probe concentration.
	The hybridization time is too short. Increase the hybridization time.
	The labeling efficiency of the hybridization probe is too low. • Check the labeling efficiency of hybridization probe (*see* the protocol above). • Prepare a new probe if the labeling efficiency is too low.
	Post-hybridization washing conditions are too stringent. Decrease the temperature of post-hybridization washes.
	The nucleic acid transfer procedure is inefficient. Increase the transfer time.
High/uneven background	**Inefficient blocking.** Prolong the blocking step.
	Inefficient washing. Prolong the washing step and/or increase the volume of the washing buffer.
	There are traces of agarose on membrane. Increase the membrane wash steps prior to the blocking step to ensure that no traces agarose gel are left on the membrane.
	The membrane does not float freely in solutions during detection procedure. Increase the volume of solutions in all steps.
	Impure water. Use only high-quality water.
	The working solution(s) have been contaminated by bacterial growth during storage. Add sodium azide to prevent bacterial contamination. Prepare new working dilutions.
	Excess probe has been applied. Reduce the probe concentration.
	The pre-hybridization procedure is inefficient. Prepare a new pre-hybridization solution using only high quality non-specific DNA. Increase the pre-hybridization time.
	Post-hybridization washes are not sufficient. Increase the temperature and/or the duration of washes.

50X BCIPT/NBT Solution

T Toxic

Hazard-determining component of labeling: **N,N-dimethylformamide**

Risk phrases

R61	May cause harm to the unborn child.
R20/21	Harmful by inhalation and in contact with skin.
R36	Irritating to eyes.

Safety phrases

S53	Avoid exposure - obtain special instructions before use.
S9	Keep container in a well-ventilated place.
S23	Do not breathe gas/fumes/vapour/spray.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S60	This material and its container must be disposed of as hazardous waste.

50X BCIPT/NBT Solution

Hazard statements:

H360D May damage the unborn child

Precautionary statements:

P280 Wear protective gloves/protective clothing/eye protection/face protection.
P281 Use personal protective equipment as required.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact
lenses, if present and easy to do. Continue rinsing.
P308+P313 IF exposed or concerned: Get medical advice/attention.
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/ international regulations..

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.