QuickProtocol™

Biotin Chromogenic Detection Kit

Detection procedure (100cm² membrane)

1 Prepare assay solutions

Blocking/Washing Buffer, Blocking Solution and Detection Buffer (*see* the reverse side: 1, 2, 4)

2 Wash membrane

- Place membrane into container
- Add 30ml of the Blocking/Washing Buffer
- Gently rock 5 minutes at room temperature, discard the liquid

3 Block membrane

- Add 30ml of the Blocking Solution
- Gently rock 30 minutes at room temperature, discard the liquid

While blocking, dilute Streptavidin-AP Conjugate (*see* the reverse side: 3)

4 Biotin-streptavidin binding

- Add 20ml of diluted Streptavidin-AP Conjugate
- Gently rock 30 minutes at room temperature, discard the liquid

5 Wash membrane

- Add 60ml of the Blocking/Washing Buffer
- Gently rock 15 minutes at room temperature
- Discard the liquid

} Repeat twice

- Add 20ml of Detection Buffer (*see* the reverse side: 4)
- Gently rock 10 minutes at room temperature, discard the liquid

During the last wash, prepare Substrate Solution (*see* reverse side: 5)

6 Develop membrane

- Add 20ml of the Substrate Solution
- Incubate at room temperature in the dark

To increase sensitivity, develop color overnight

7 Stop reaction

- Discard the Substrate Solution
- Add 30ml of either Milli-Q or double deionized water, rinse for few seconds
- Discard the liquid, drain and air-dry the membrane

Do not dry the membrane if stripping and re-hybridization of the membrane are planned

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Volumes of assay solutions required (100cm² membrane)

Assay Solution	Required volume
Blocking/Washing Buffer	200ml
Blocking Solution	50ml
Diluted Streptavidin-AP Conjugate	20ml
Detection Buffer	40ml
Substrate Solution	20ml

Note. Scale the volumes of solutions according to the size of your membrane and container to ensure that the membrane floats freely and is evenly covered with the solution.

Preparation of Assay Solutions

1. Blocking/Washing Buffer.

Dilute 20ml of 10X Blocking/Washing Buffer with 180ml of either Milli-Q or double deionized water.

Diluted Blocking/Washing Buffer can be stored at +4°C for one week.
Add Sodium Azide (final concentration 0.1%) for a longer storage.

2. Blocking Solution.

Dissolve 0.5g of Blocking Reagent in 50ml of the Blocking/Washing Buffer.
Agitation at 50-60°C is recommended.

Store Blocking Solution in aliquots at -20°C.

3. Diluted Streptavidin-AP Conjugate.

Add a 4µl aliquot of concentrated Streptavidin-AP Conjugate to 20ml of the Blocking Solution just prior to use.

4. Detection Buffer.

Dilute 4ml of 10X Detection Buffer with 36ml of either Milli-Q or double deionized water.

Diluted Detection Buffer can be stored at +4°C for one week.
Add Sodium Azide (final concentration 0.1%) for a longer storage.

5. Substrate Solution.

Add a 0.4ml aliquot of 50X BCIP/NBT Solution to 20ml of the Detection Buffer just prior to use.

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