Instruction manual PerfectHyb hybridization solution 0810

F1017K

PerfectHyb hybridization solution

HYB-101 250 ml
Store at room temperature

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CAUTION
All reagents in this kit are intended for research purposes. Do not use for diagnostic or clinical purposes. Please observe general laboratory precautions and safety measures while using this kit.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

[1] Introduction

Description

PerfectHyb is an easy-to-use hybridization solution which contains a rate enhancer. This reagent has been optimized for Northern and Southern blot analyses and includes the following features:

Features

-Reduced hybridization time, from the customary 12-24 hours to 1-2 hours
-Allows for the use of radioactive and non-radioactive nucleic acid probes
-Enhanced signals using Northern blot analysis
-Same temperature for hybridization and washing steps
-No requirement for salmon sperm DNA
-Low viscosity allows for easy handling

[2] Applications

Northern blot

-cDNA, cRNA, and oligonucleotide probes can be used for Northern blot analysis.
-Radioactive cDNA or oligonucleotide probes are recommended for analyses using commercially-available pre-blotted membranes (mRNA blotted membranes)*. Please read this instruction manual prior to use to prevent unexpected background signals, in particular with non-radioactive methods.

*This reagent has been evaluated using commercially-available pre-blotted membranes.

Southern blot

-Radioactive, as well as non-radioactive, DNA and oligonucleotide probes are recommended for Southern blot analysis. Please read this instruction manual prior to use to prevent unexpected background signals, in particular with non-radioactive methods.

[3] Principle

This reagent reduces hybridization time due to a rate enhancer included in the solution. On Northern blots, this reagent also enhances signal intensity.

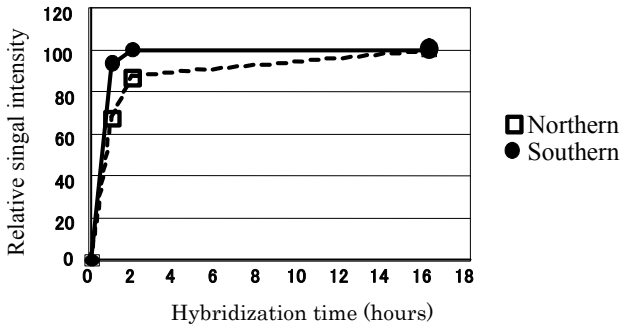

Fig. 1 Relationship between hybridization time and relative signal intensity on Northern and Southern blots using DIG-labeled β-actin and VNTR probes.

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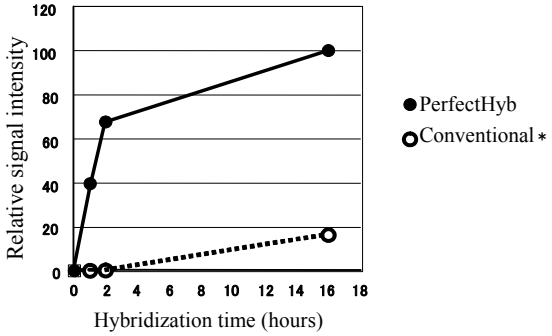

Fig. 2 Relationship between hybridization time and relative signal intensity when detecting a rarely expressed mRNA (transferrin receptor) by Northern blot. .

[4] Components

This reagent contains the following components:

PerfectHyb hybridization solution 250 ml

*This product can be stored at room temperature. In the case of long-term storage, this product should be stored at 2-8°C. At ≤ 15°C, a precipitate may form. If this occurs, the solution should be heated to 37°C and thoroughly mixed to completely dissolve precipitate.

[5] Materials required

The following materials are required, but not supplied:

(1) Reagents

-Wash solution A
2x SSC (pH 7.0), 0.1% SDS

-Wash solution B*
0.1x SSC (pH 7.0), 0.1% SDS

*This reagent is required for Southern blots with all probes, as well as Northern blots using a RNA probe (see [7] Reagent).

(2) Instruments

-Heating bath with shaker, or hybridization oven
-Heat sealer (when using hybridization bag)
-Hybridization bag (optional)
-X-ray film (optional)

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[6] Protocol

1. Northern and Southern blot analyses using cDNA and cRNA probes

(1) Preparation of probes

Probe labeling should be performed according to the instruction manual. The free nucleotides should be removed prior to use.

NOTES

-The specific activity of radioactive probes should be confirmed prior to use. The radioactive probes should be labeled just before use.

-Non-radioactive probes should be confirmed by a spot test, in accordance with the instruction manual.

-Probes with direct repeats, or long probes (≥4 kb), tend to generate unexpected extra bands or background signals. Probe sequences should be confirmed prior to preparation.

(2) Hybridization and washing conditions

The following table provides hybridization and washing conditions.

	Probe	Label	Hybridization	Wash Solution 1	Wash Solution 2
Time	-	-	1h - overnight	5 minutes x 2	15 minutes x 2
Northern Blot	DNA	RI Non-RI	68°C	68°C (A)	68°C (A)
	RNA	Non-RI	68°C	68°C (A)	68°C (B)
Southern Blot	DNA	RI Non-RI	68°C	68°C (A)	68°C (B)

(A): Wash solution A, (B): Wash solution B

NOTES

-When using a heating bath for hybridization, hybridization should be performed in a hybridization bag. The membranes should be washed with shaking using a container.

-A hybridization oven is suitable for the analyses with radioactive probes. In this case, membranes should be washed in the hybridization oven.

(3) Probe concentration

The following table lists recommended probe concentrations. Higher-concentrated probes tend to generate background signals, in particular when employing non-radioactive probes.

Label	Probe	Probe concentration
RI	DNA	1–2 x 10^6 cpm/ml or 1–10 ng/ml
Non-RI	DNA	0.2–1 ng/ml
	RNA	

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(4) Hybridization time

The following table lists hybridization times.

	Expression level of target mRNA	Hybridization time
Southern blot	-	1 hour
Northern blot	High	1 hour
	Medium	2 hours
	Low	2 hours–overnight
When using long probes (≥ 4 kb)		Overnight*

*Long probes (≥ 4 kb) tend to generate background signals. Overnight hybridization reduces background levels when using the long probes (≥ 4 kb). Probe concentration should be reduced on analyses with long (≥ 4 kb), non-radioactive probes.

NOTES

-Probe concentration and hybridization time should be determined according to the probe concentration and expression levels of the target genes.

-Overnight hybridization with a radioactive probe is recommended for detection of mRNA with low or unknown expression levels.

(5) Protocol

(a) Prehybridize 10 x 10 cm (100 cm^2) membranes in a minimum volume of 5 ml PerfectHyb hybridization solution at 68°C for 20 minutes.

(b) Denature labeled probe at 100°C for 5 minutes*.

*Labeled probes should be denatured in low-salt water (e.g., distilled water or TE buffer) just prior to use.
*RNA probes should be denatured prior to use.

(c) In the case of 100 cm^2 membranes, add the denatured probe to a minimum volume of 5 ml, pre-warmed, fresh PerfectHyb hybridization solution**.

**Assure that probe is thoroughly mixed with fresh hybridization solution.

(d) Replace pre-hybridization solution with fresh hybridization solution containing the labeled probe.

(e) Incubate at 68°C for 1 hour–overnight.

(f) Wash membrane two times in pre-warmed Wash solution 1 at 68°C for 5 minutes.

(g) Wash membrane two times in pre-warmed Wash solution 2 at 68°C for 15 minutes.

(h) [Radioactive probe]
Transfer membrane with forceps onto filter paper. Then, follow with exposure to X-ray film at -70°C.

[Non-radioactive probe]
Perform detection according to the instruction manual.

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2. Analysis using oligonucleotide probes

(1) Preparation of probes

Probes should be labeled according to instruction manual. The free nucleotides should be removed prior to use. Probe labeling efficiency should be checked prior to use.

(2) Calculation of Tm value of probes

The Tm value of each probe should be determined according to the following equation:

(a) Oligonucleotide probe (< 18 base)

$$Tm = [(A+T) \times 2°C] + [(G+C) \times 4°C]$$

(b) Oligonucleotide probe (≥ 18 base)

$$Tm = 81.5 + 16.6 (\log 10 [Na+]) + 0.41(\%G+C) – (600/N)$$

*A: the number of adenine bases in the oligonucleotide
T: the number of thymine bases in the oligonucleotide
G: the number of guanine bases in the oligonucleotide
C: the number of cytosine bases in the oligonucleotide
%G+C: percentage of (G+C) in the oligonucleotide
N: the number of nucleotides in the oligonucleotide
[Na+]: 0.75 M

(3) Hybridization and washing conditions

The following table lists hybridization and washing conditions.

	Hybridization	Wash Solution 1	Wash Solution 2
Time	1-2 hours	5 minutes x 2	10 minutes x 2
Radioactive probe	Tm–10°C	Tm–10°C (A)	Tm–10°C (A)
Non-radioactive probe			

(A): Wash solution A

NOTES

-When using probes with a high Tm (≥ 70°C), hybridization should be performed at 55-60°C.

-When using a heating bath for hybridization, hybridization should be performed in a hybridization bag. The membranes should be washed with shaking using a container.

-A hybridization oven is suitable for analyses with radioactive probes. In this case, the membrane should be washed in the hybridization oven.

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(4) Probe concentration

The following table lists recommended probe concentrations. Higher-concentrated probes tend to generate background signals, in particular with analyses employing non-radioactive probes.

Label	Probe concentration
RI	2.5–5 pmoles/ml
Non-RI	0.5–1 pmoles/ml

(5) Protocol

(a) Prehybridize 10 x 10 cm membranes in a minimum total volume of 5 ml of PerfectHyb hybridization solution at optimal hybridization temperature for 20 minutes.

(b) Add the labeled probe to 5 ml of prewarmed, fresh PerfectHyb hybridization solution*.

*Assure that probe is thoroughly mixed with fresh hybridization solution.

(c) Replace pre-hybridization solution with fresh hybridization solution containing the labeled probe.

(e) Incubate at optimal hybridization temperature for 1–2 hours.

(f) Wash membrane two times in pre-warmed Wash solution 1 at the optimal temperature for 5 minutes.

(g) Wash membrane two times in pre-warmed Wash solution 2 at the optimal temperature for 10 minutes.

(h) [Radioactive probe]
Transfer membrane with forceps to filter paper. Then, follow with exposure to X-ray film at -70°C.

[Non-radioactive probe]
Perform detection according to the instruction manual.

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[7] Reagents

1. 20x SSC [3 M NaCl, 0.3 M Sodium citrate] (1000 ml)

175 g NaCl
88 g tri-sodium citrate dihydrate
Dissolve in 900 ml distilled water
Adjust pH to 7.0 with 1N HCl
Adjust volume to 1000 ml with distilled water
Store at room temperature

2. 10% SDS (500 ml)

50 g SDS
Adjust volume to 500 ml with distilled water
Store at room temperature

3. Wash solution A [2x SSC, 0.1% SDS] (500 ml)

50 ml 20x SSC
5 ml 10% SDS
Adjust volume to 500 ml with distilled water
Store at room temperature

4. Wash solution B [0.1x SSC, 0.1% SDS] (500 ml)

2.5 ml 20x SSC
5 ml 10% SDS
Adjust volume to 500 ml with distilled water
Store at room temperature

5. 20 x SSPE [3 M NaCl, 173 mM sodium dihydrogen phosphate, 25 mM EDTA] (1000 ml)

175 g NaCl
27 g sodium dihydrogen phosphate dihydrate
7.4 g EDTA·2Na
Dissolve in 800 ml distilled water
Adjust pH to 7.4 with 5 N NaOH
Adjust volume to 1000 ml with distilled water
Store at room temperature

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[8] Related protocol

1. Stripping of membranes

The following protocol is a typical stripping method for membranes following analysis with radioactive probes.

NOTES

-Membrane stripping tends to decrease signals with the subsequent blot. Therefore, the membrane should be baked, followed by UV irradiation cross-linking, prior to re-use.

-When using commercially available pre-blotted membranes (mRNA-blotted membranes), the membrane should be stripped according to the corresponding instruction manual.

-If the membrane has an indication concerning the stripping method, stripping should be performed according to that indication.

-Stripping efficiency may depend on the types of probes.

(1) Materials required

(a) Stripping reagent (100 ml)*

55 ml formamide
10 ml 20x SSPE
5 ml 10% SDS
Adjust volume to 100 ml with distilled water

*This solution should be prepared just prior to use.

(b) Wash solution B [0.1x SSC (pH 7.0), 0.1% SDS]
-> see [7] reagent

(c) Hybridization oven or heating bath

(2) Protocol

(a) Transfer the membrane to a hybridization bath or bottle.

(b) Add 10 ml of stripping solution to the 10 cm x 10 cm (100 cm^2) membrane.

(c) Incubate the membrane at 68°C for 1-2 hours.

(d) Discard stripping solution and wash membrane at 68°C for 10 minutes.

Notes

-The radioactivity of stripped membranes should be measured with a survey counter or X-ray film. When using X-ray film, exposure should be performed overnight.
-Upon stripping, the membrane should be stored properly to prevent drying.

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2. Purification of radioactive probes

The following protocol is a simple purification method for radioactive cDNA probes using a DNA fragment purification kit (e.g., MagExtractor -PCR & Gel clean up-).

(1) Materials required

-MagExtractor -PCR & Gel clean up- (Code No. NPK-601)
-75% Ethanol
-Magnetic stand (e.g., Magical trapper [Code No. MGS-101])

(2) Protocol

(a) Dispense ≤ 50 µl of labeled DNA probe into a 1.5-ml microtube.

(b) Add 200 µl **Binding Solution**.

(c) **[Binding]** Add 15 µl **Magnetic Beads** and vortex the tube every 10 seconds for 1-2 minutes.

Notes
Completely resuspend the magnetic beads prior to use.

(d) Place the tube in the magnetic stand. The magnet will attract the magnetic beads, separating then from the specimen solution.

Fig. 1
Magnetic separation

(e) Upon magnetic capture, carefully remove the supernatant.

(f) **[Washing]** Add 300 µl **Washing Solution** to the beads and vortex for 10 seconds.

(g) Place the tube in the magnetic stand, and collect the beads with the magnet.

(h) Upon magnetic capture, carefully remove the supernatant and discard into a waste tank.

(i) **[Washing]** Add 1 ml **75% EtOH** to the tube and vortex for 10 seconds.

(j) Place the tube in the magnetic stand, and collect the beads with the magnet.

(k) Upon magnetic capture, carefully remove the supernatant.

Notes
The 75% EtOH should be completely removed after flash centrifugation.

(l) **<Elution>** Add 25-50 µl **sterilized water** and mix well for 10 seconds.

(m) Incubate at room temperature for 2 minutes.

(n) Place the tube in the magnetic stand after briefly vortexing.

(o) Collect the supernatant and place into a fresh tube.

Notes
-Handling should be performed opposite an acrylic board.
-1.5 ml microtubes with screw-caps should be used to prevent contamination.

[9] Troubleshooting

Symptom	Cause	Solution
Hybridization signals absent or very week	Insufficient exposure	Prolong exposure time.
	Specific activity or labeling efficiency of probe was too low	Confirm specific activity or labeling efficiency of probes.
	Probe concentration was too low	Increase probe concentration.
	Labeled probe was not fresh	When using radioactive probes, the probe should be labeled just prior to use. Do not store the probe.
	Labeled probe was not denatured	Denature the probes just prior to use.
	Insufficient hybridization time	Overnight hybridization is effective for detecting low-expressing mRNA on Northern blot.
	Reprobing	Reprobing decreases signal intensity. Prolong the exposure time.
	Too much washing	Reduce the washing time. Increasing SSC concentration (e.g., 0.1x SSC -> 0.2x SSC) may enhance the signal.
	Blotted nucleic acids were not sufficient	Increase the amount of nucleic acids on the membrane.
High background signals	Too much labeled free nucleic acid	Remove the labeled free nucleic acids prior to use.
	Probe concentration was too high	Decrease probe concentration. High-concentration probes tend to generate high background signals.
	Length or sequence of the probe was not appropriate	Probes prepared from a long template (≥ 4 kb) or possessing repeat sequences tend to generate non-specific signals. Reconfirm the template size and sequence.
	Drying of the membrane at hybridization steps	Drying of the membrane at the hybridization step increases background signals. Take care that the membrane does not become dry.
	Foaming of the hybridization solution in the hybridization bag	Foaming of the hybridization solution causes irregular spot signals. Remove bubbles from the hybridization bag prior to hybridization.
	Insufficient washing solution	Use sufficient volumes of washing solution.
	Detection method was not inappropriate	Non-radioactive probes tend to generate high background signals. When using non-radioactive probes, the detection should be performed according to the instruction manual.
Extra bands	The probe contained non-specific sequences	Direct repeats (e.g., Alu) in the probe sequence generate extra bands. Additional bands may be reduced by including denatured salmon sperm DNA (final concentration: 100 µg/ml).
	Insufficient washing	Increasing washing steps or decreasing SSC concentration (e.g., 1x SSC -> 0.5x SSC) may eliminate the extra bands.

[10] Related products

Product name	Package	Code No.
MagExtractor -PCR & Gel Clean up-	1 kit	NPK-601
Magnetic stand **Mag*ical Trapper***	1 piece	MGS-101

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