Northern Bound™ Multiple Tissue Total RNA
Northern Blot, Human-Alzheimer's Disease

Product Number **N 5534, N 5659, N 7409, and N 7534**

ProductInformation

TECHNICAL BULLETIN

Product Description

Northern Bound™ Multiple Tissue Northern Blots contain high quality total RNA isolated from individual tissues (see Table 1). 20 µg of each RNA is subjected to electrophoresis, transferred to a positively charged nylon membrane, and UV crosslinked.

The upper left corner of the blot (next to the origin of lane 1) has been cut off to provide orientation. The molecular weight marker positions have been marked by dots.

Material Provided

- Northern Bound™ Multiple Tissue Total RNA Northern Blot, Human-Alzheimer's Disease 1 Blot

Reagents Required but Not Provided

(Product codes have been given where appropriate)

- 20x SSC (3 M sodium chloride, 0.3 M sodium citrate, pH 7.0), Product Codes S 6639, S 8015, or S 0902
- 10% SDS solution (Product Code L 4522)
- PerfectHyb™ Plus (Product Code H 7033)

Note: The following products as a group may be used in place of PerfectHyb Plus:

- Denhardt's solution (Product Codes D 2532 or D 9905) **or**
  Denhardt's solution prepared from Ficoll® (Product Code F2637), polyvinylpyrrolidone (Product Code P 5288) and BSA (Product Code B 2518)
- 0.5 M EDTA, pH 8.0 (Product Code E 7889)
- Sodium phosphate, monobasic (Product Code S 3139)
- Sodium phosphate, dibasic (Product Code S 3264)
- protectRNA™ RNase inhibitor, 500x (Product Code R 7397)
- DNA from salmon testes, denatured (Product Codes D 9156 or D 7656)

Precautions and Disclaimer

Sigma's Northern Bound™ Multiple Tissue Total RNA Northern Blots are for R&D use only, not for drug, household, or other uses.

Storage

Store the blots at room temperature, protected from light.

Table 1.
Total RNA location for the multiple tumor Northern blots

Product. No.	N 5534	N 5659	N 7409	N 7534
Total RNA Source Tissue	Alzheimer's	Alzheimer's	Normal Control	Normal Control
Lane 1	precentral gyrus	frontal lobe	precentral gyrus	frontal lobe
Lane 2	postcentral gyrus	temporal lobe	postcentral gyrus	temporal lobe
Lane 3	frontal lobe	parietal lobe	frontal lobe	parietal lobe
Lane 4	temporal lobe	occipital lobe	temporal lobe	occipital lobe
Lane 5	parietal lobe	pons	parietal lobe	pons
Lane 6	occipital lobe	thalamus	occipital lobe	thalamus
Lane 7	cerebellum	corpus callosum	cerebellum	corpus callosum

Preparation Instructions

Buffer preparation

- Hybridization buffer: Use either Sigma's PerfectHyb Plus or a hybridization buffer prepared with the following:

5X	SSC
1% (w/v)	SDS
5X	Denhardt's solution
5 mM	EDTA, pH 8.0
50 mM	Na phosphate buffer, pH 7.5
1X	protectRNA
100 µg/ml	DNA from salmon sperm (Boil the DNA for 10 minutes to denature before adding to other components.)

  Store hybridization buffer at –20 °C.

- Low stringency wash buffer: 2X SSC with 0.1% (w/v) SDS. To 500 ml of molecular biology grade water, add 100 ml of 20X SSC stock solution and 10 ml of 10% (w/v) SDS stock solution. Dilute to 1 L with water.

- High stringency wash buffer: 0.5X SSC with 0.1% (w/v) SDS. To 500 ml of molecular biology grade water, add 25 ml of 20X SSC stock solution and 10 ml of 10% (w/v) SDS stock solution. Dilute to 1 L with water.

- Ultra-high stringency wash buffer: 0.1X SSC with (w/v) 0.1% SDS. To 500 ml of molecular biology grade water, add 5 ml of 20X SSC stock solution and 10 ml of 10% (w/v) SDS stock solution. Dilute to 1 L with water.

- 50X Denhardt's solution (if not using a commercial preparation):

1% (w/v)	Ficoll®
1% (w/v)	Polyvinylpyrrolidone
1% (w/v)	BSA

  Store at –20 °C.

Procedure

Hybridization Procedure

Optimal results are obtained only if the following procedure is precisely followed. The use of other hybridization buffers may give poor results. A blocking agent is not necessary when using PerfectHyb Plus. If a blocking agent is preferred, Sigma suggests using 0.1 mg/ml of single stranded DNA.

1. Prepare probe DNA using the random oligonucleotide primers method[1] or a random-prime kit (see related products section). If using a radioactive probe the specific activity of the probe should be >5 x 10^8 cpm/µg.

2. Carry out hybridization in a hybridization tube or a sealed plastic bag with constant agitation. Prehybridize the blot in a minimum of 6 ml of hybridization buffer at 42 °C for at least 5 minutes if using PerfectHyb Plus or for 3 hours if using the prepared hybridization buffer.

3. Denature double stranded DNA probes at 100 °C for 5 minutes. Quick chill on ice for 2 minutes. Single stranded DNA and RNA probes do not require denaturation prior to addition to the hybridization reaction.

4. Add 1 to 2 x 10^6 cpm labeled DNA probe per ml of hybridization solution. Avoid direct contact of the concentrated probe with membrane.

5. Hybridize the blot at 42 °C with constant agitation for 1 to 3 hours if using PerfectHyb Plus or 16 to 24 hours if using the prepared hybridization buffer.

6. Remove the hybridization solution and wash the blot at room temperature 3 to 4 times with a minimum of 50 ml of low stringency wash buffer for 10 minutes each.

7. Wash the blot twice at 50 °C with a minimum of 50 ml of high stringency or ultra-high stringency wash buffer for 20 minutes each.

8. Wrap the damp blot with plastic wrap and detect probe binding using standard autoradiographic, phosphorimaging, or direct imaging techniques. Autoradioradiography and phosphorimaging may require empirical determination of optimal exposure time. Making a few exposures for different lengths of time is recommended.

   Note: Do not allow the blot to dry, as this will prohibit probe removal from the blot thereby preventing reprobing.

Probe Removal

The probe can be removed after exposure to film and the blot can be reused at least 3 times if the following procedure is followed.

1. Heat approximately 500 ml of 0.5% (w/v) SDS in a large beaker to boiling.

2. Remove the blot from the plastic wrap and immediately place it into the boiling SDS solution. Minimize exposure of the blot to air.

3. Carefully remove the beaker from the heat source and gently agitate the solution for 10 minutes.

4. Remove the blot from the hot SDS solution and wash the blot at room temperature for 5 minutes in 2x SSC.
5. Wrap and seal the damp blot in plastic wrap and store at –20 °C until next use.

Troubleshooting Guide

Problem	Cause	Solution
High background	Wrong hybridization buffer recipe	Use either PerfectHyb Plus or the specified hybridization buffer.
	Non-specific binding of probe to target nucleic acids	Add heat denatured, sheared, salmon testes DNA to a final concentration of 100 µg/ml to both pre-hybridization and hybridization buffers.
	Exposure to film too long	Shorten exposure time.
	Concentration of enzyme conjugate in non-radioactive detection is too high	Dilute the enzyme conjugate further. The specific dilution required for optimal signal to noise ratio must be determined empirically.
	Amount of radioactive probe too high	Do not use more than 2×10^6 cpm/ml.
Weak or absent signal	Probe was not labeled efficiently	Check that the specific activity of the labeled probe is $>5 \times 10^8$ cpm/µg. For non-radioactive probes, check the incorporation of the hapten by spotting and detecting serial dilutions of probe in direct comparison to a known standard. If probes are not labeled properly, remake and confirm adequate incorporation rate.
	Probe not homologous	If the probe being used is from another species, it may be necessary to reduce the stringency of the final wash. Use high stringency wash buffer; do not use ultra-high stringency wash buffer. Reduce the final wash temperature.
	Signal has decreased due to repeated stripping and reprobing	The signal will decrease approximately 20% with each probing and stripping cycle using the above procedure. Perform hybridization for rare or unknown genes in the first cycles of the blot and of control and highly abundant genes in the later cycles.

Related Products

Product Name	Product Code
Mouse Multiple Tissue Northern Blot,	BLOT-2
Mouse Embryo Multiple Developmental Stages Northern Blot	BLOT-3
PerfectHyb Plus	H 7033
All-in-One Nick Translation Labeling Mix –dCTP	N 9155, N 8530
All-in-One Random Prime Labeling Mix –dCTP	R 7522, R 9647
All-in-One Random Prime Labeling Mix –dATP	R 7022, R 9522
All-in-One NickTranslation Labeling Mix –dATP	N 8405, N 9280

References

1. Sambrook, J. *et al.*, Molecular Cloning - A Laboratory Manual p. 10.13, Cold Spring Harbor Press (1989).
2. Ausubel, F.M., *et al.*, Short Protocols in Molecular Biology. John Wiley and Sons, Inc., USA (1995).

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