



Product Information

Northern Bound™ Multiple Tissue mRNA Northern Blot, Human

Product Codes **N 5784, N 5909, N 6034, N 6159, and
N 6284**

TECHNICAL BULLETIN

Product Description

Northern Bound™ Multiple Tissue Northern Blots contain high quality poly A⁺ RNA isolated from individual tissues (see Table 1). Three µg of each mRNA is subjected to electrophoresis on a denaturing (formaldehyde) 1% agarose gel, 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 mRNA
Northern Blot, Human 1 Blot

Reagents Required but Not Provided

(Product Codes have been given where appropriate)

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

- DNA from salmon testes, denatured (Product Codes D 9156 or D 7656)
- 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 F 2637), 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)

Precautions and Disclaimer

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

Storage/Stability

Store blots at room temperature, protected from light.

Table 1.

mRNA location for the multiple tissue Northern blots

Product No.	N 5784	N 5909	N 6034	N 6159	N 6284
	mRNA Source Tissue	mRNA Source Tissue	mRNA Source Tissue	mRNA Source Tissue	mRNA Source Tissue
Lane 1	Heart	Brain	Frontal Lobe	Stomach	Uterus
Lane 2	Brain	Uterus	Temporal Lobe	Jejunum	Cervix
Lane 3	Liver	Placenta	Parietal Lobe	Ileum	Ovary
Lane 4	Pancreas	Kidney	Occipital Lobe	Colon	Testis
Lane 5	Skeletal Muscle	Spleen	Cerebellum	Rectum	Prostate
Lane 6	Lung	Lung	Lung	Lung	Lung

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	Sodium 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 0.1% (w/v) 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.

- Prepare probe DNA using the random oligonucleotide primers method¹ or a random-prime kit (see related products section). If using a

radioactive probe, the specific activity of the probe should be $>5 \times 10^8$ cpm/µg.

- 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.
- 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.
- Add 1 to 2×10^6 cpm labeled DNA probe per ml hybridization solution. Avoid direct contact of the concentrated probe with membrane.
- 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.
- 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.
- 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.
- Wrap the damp blot with plastic wrap and detect probe binding using standard autoradiographic, phosphorimaging, or direct imaging techniques. Autoradiography 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.

- 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 $\mu\text{g/ml}$ 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 the 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

<u>Product Name</u>	<u>Product Code</u>
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 Nick Translation 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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DEC/MAM 11/01

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