MULTIPLE TISSUE NORTHERN BLOT, RAT

A. ProductInformation

Product No. **BLOT-1**
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TECHNICAL BULLETIN

Product Description
Sigma's Rat Multiple Tissue Northern Blot contains polyA$^+$ RNA isolated from 10 different organs from Sprague Dawley rats (1. brain, 2. heart, 3. liver, 4. kidney, 5. spleen, 6. testis, 7. lung, 8. thymus, 9. placenta, and 10. upper thigh muscle). 2 µg of each mRNA is subjected to electrophoresis on a 1.2% agarose gel in the presence of formaldehyde. The mRNA is then transferred to a positively charged nylon membrane and UV crosslinked. The blot is supplied stained with Blot Stain Blue (Product No. B1177) to ensure the quality of the transfer.

The upper left corner (next to the origin of lane 1) has been cut off to provide orientation. The origin of all lanes has been marked by an ink dot. The position of the molecular weight markers has been also marked by ink dots.

The polyA$^+$ RNAs used in the blot were tested for integrity and purity by gel electrophoresis. The blots are first tested visually for the quality of the transfer by monitoring the Blot Stain Blue staining. This allows Sigma to guarantee accurate transfer of the mRNA. One blot from every lot is tested by hybridization to rat GAPDH probe.

The rat GAPDH control probe provided is a 1.35 kb cDNA fragment (Genbank accession number X02231). The amount of probe DNA is determined by absorbance measurement at 260 nm and size is determined by gel electrophoresis.

Reagents Provided
- Northern Blot, Rat Multiple Tissue, Product No. N2033 — 1 each
- GAPDH cDNA control probe, Product No. G0789 — 0.1 µg

Reagents Required but Not Provided
(Sigma product numbers have been given where appropriate)
- 20X SSC (3 M sodium chloride, 0.3 M sodium citrate, pH 7.0), Product Nos. S6639, S8015 or S0902
- 10% SDS solution, Product No. L4522
- DNA from salmon testes, denatured, Product Nos. D9156 or D7656
- PerfectHyb Plus, Product No. H7033
  Note: The following products as a group may be used in place of PerfectHyb Plus.
- Denhardt's solution, Product Nos. D2532 or D9905
  or
  Denhardt's solution prepared from Ficoll® (Product No. F2637), polyvinylpyrrolidone (Product No. P5288) and BSA (Product No. B2518)
- 0.5 M EDTA, pH 8.0, Product No. E7889
- Sodium phosphate, monobasic, Product No. S3139
- Sodium phosphate, dibasic, Product No. S3264
- protectRNA RNase inhibitor, 500X, Product No. R7397

Precautions and Disclaimer
Sigma's Rat Multiple Tissue Northern Blot is for laboratory use only. Not for drug, household or other uses.

Storage
Store all kit components at -20°C and protect from light.

General remarks:

1. Probing of Sigma's Rat Multiple Tissue Northern Blot has been optimized using PerfectHyb Plus hybridization buffer (Product No. H7033). Sigma advises using this buffer, but this blot may also be probed using more common techniques.
2. The blue stain will come off during the hybridization procedure, therefore it is recommended to first photograph or photocopy the blot for your records before starting hybridization.
3. Handle the blot with powder-free gloves and clean forceps only. Avoid touching the blot where RNA is present.

Figure 1.

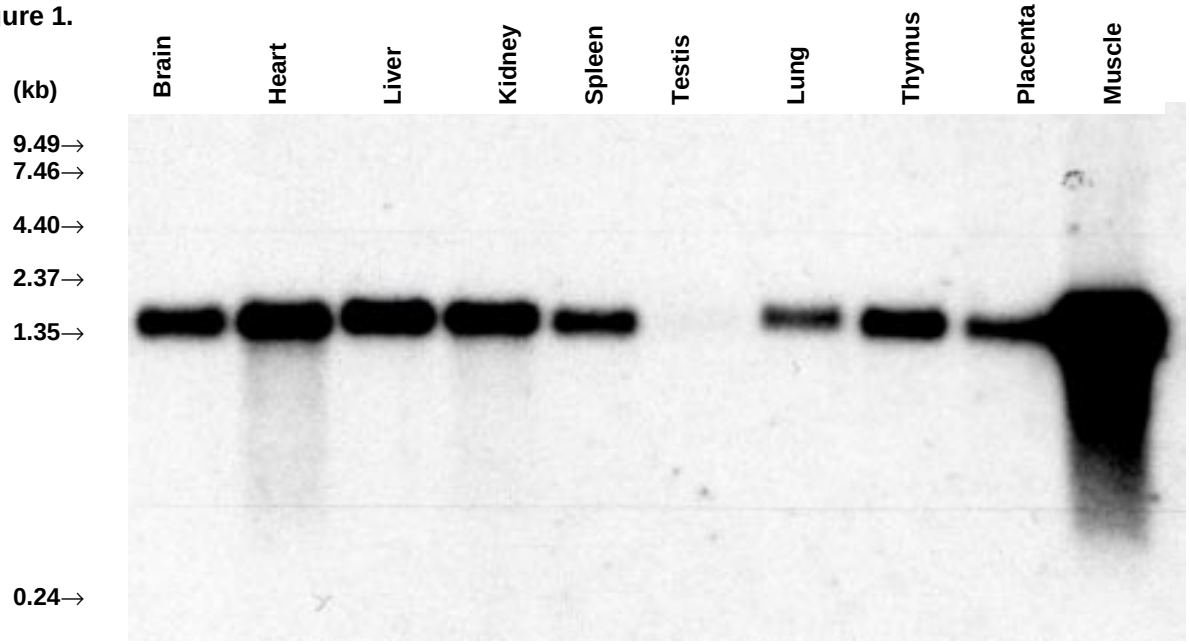

Figure 1. GAPDH Expression Levels in a Rat Multiple Tissue Northern Blot
A ^{32}P-dCTP labeled GAPDH DNA probe was prepared by random primed labeling and hybridized to a rat Multiple Tissue Northern Blot (BLOT-1). Hybridization was carried out with PerfectHyb™ Plus (H7033) supplemented with 0.1 mg/ml sheared salmon sperm DNA (D9156) at 65°C for 1-2 hours and imaged by exposure to film for 2 hours.

Preparation Instructions

Buffer preparation

- Hybridization buffer: Use either Sigma's PerfectHyb Plus or the following Hybridization Buffer.

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, 0.1% SDS
  To 500 ml molecular biology grade water, add 100 ml of 20X SSC stock solution and 10 ml 10% SDS stock solution. Bring up to 1 L with water.
- High stringency wash buffer: 0.5X SSC, 0.1% SDS
  To 500 ml molecular biology grade water, add 25 ml of 20X SSC stock solution and 10 ml 10% SDS stock solution. Bring up to 1 L with water.
- Ultra-high stringency wash buffer: 0.1X SSC, 0.1% SDS
  To 500 ml molecular biology grade water, add 5 ml of 20X SSC stock solution and 10 ml 10% SDS stock solution. Bring up to 1 L with water.

- 50X Denhardt's solution (if not purchasing Denhardt's solution)
  1% Ficoll
  1% Polyvinylpyrrolidone
  1% BSA
  Store at -20°C.
- GAPDH cDNA control probe: Reconstitute with 20 µl sterile water to give a solution of 5 ng/µl in 10 mM Tris HCl, 1 mM EDTA, pH 8.

Procedure

Hybridization Procedure

Results are guaranteed only if the following procedure is precisely followed. The use of other hybridization buffers may give poor results. Sigma has found that 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]. If using a radioactive probe the specific activity of the probe should be >$5x10^8$ cpm/µg.

2. Carry out hybridization in a hybridization tube or a sealed plastic bag with constant agitation. Prehybridize the blot in minimum of 6 ml of hybridization buffer at 65-68°C for 3-6 hours if using the above hybridization buffer or for at least 5 minutes if using PerfectHyb Plus.

3. For double stranded DNA probes, denature by heating probe to 100°C for 5 minutes. Quick chill on ice 2 minutes. Single strand DNA and RNA probes do not require denaturation prior to addition to the hybridization reaction.

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

5. Hybridize the blot at 65-68°C with constant agitation for 16-24 hours if using the above hybridization buffer or for 1-3 hours if using PerfectHyb Plus.

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

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

8. Wrap the damp blot with plastic wrap and expose it to film using standard autoradiography techniques. Making a few exposures for different lengths of time is recommended.
   NOTE: Do not allow the blot to dry, as that will prevent removal of the probe from the blot and prevent future reuse of the blot.

Removal of Probe

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

1. Heat approximately 500 ml of 0.5% SDS in a large beaker to boiling.

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

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

4. Wash the blot in 2X SSC for 5 minutes at room temperature.

5. Wrap and seal the damp blot in plastic wrap. Store the blot 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 $2x10^6$ cpm/ml.
Weak or absent signal	Probe was not labeled efficiently	Check that the specific activity of the labeled probe is $>5x10^8$ 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 approx. 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.

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)

Related Products

Mouse Multiple Tissue Northern Blot, Product No. BLOT-2
Mouse Embryo Multiple Developmental Stages Northern Blot, Product No. BLOT-3
PerfectHyb Plus, Product No. H7033
All-in-One Nick Translation Labeling Mix –dCTP, Product Nos. N9155, N8530
All-in-One Nick Translation Labeling Mix –dATP, Product Nos. N8405, N9280
All-in-One Random Prime Labeling Mix –dCTP, Product Nos. R7522, R9647
All-in-One Random Prime Labeling Mix –dATP, Product Nos. R7022, R9522

Please see reverse side of the invoice or packing slip.