

Sample Preparation for RT-PCR

For brain tissue: start with 10-100 mg tissue

RNA Prep

Notes: Use 2054 (5 ml) tubes for homogenization. Do not spin these faster than 9000 rpm.

Follow Qiagen's RNeasy Lipid Tissue Mini Kit including optional DNase I treatment. Quantitate samples. (Remove 1 μ l/sample before freezing in liquid N₂).

cDNA Prep

RT Reaction Mix (per AB protocol): Set up one +RT and one -RT as control (per sample)

	Volume	Final Concentration
RNA	1 to 2 μ g	
10X TaqMan RT Buffer	10.0 μ l	1X
25 mM MgCl ₂	22.0 μ l	5.5 mM
dNTPs mix	20.0 μ l	500 μ M per dNTP
*Oligo d(T) ₁₆	5.0 μ l	
RNase Inhibitor	2.0 μ l	4.0 U/ μ l
Multiscribe Reverse Transcriptase (50 U/ μ l)	2.5 μ l	1.25 U/ μ l
RNase-free water	to 100 μ l	

*(Random hexamers, oligo d(T) or sequence-specific primers can be used for cDNA synthesis.)

Thermal cycling: (1) 25°C for 10 min (incubation), (2) 48°C for 30 min (reverse transcription), (3) 95°C 5 min (reverse transcriptase inactivation).

Purifying cDNA

Use Microcon YM-30 centrifugal filter devices (Millipore).

Put column in tube (provided). Add cDNA to column (100 μ l).

Add 400 μ l H₂O to column.

Spin in Tomy at 12,000 rpm for 15 min at 4°C.

Discard flow through.

Wash 2x with 500 μ l H₂O as above for 15 min/4°C.

Add 500 μ l H₂O. Spin 12,000 rpm for 10 min/4°C.

Carefully turn column over into new collection tube.

Spin at 12,000 rpm for 15 min to elute.

Adjust volume to equalize samples (i.e., make sure all samples have ~100 μ l). Store at -20°C.