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Rat Brain Tissue RNA Extraction/cDNA Synthesis for qPCR

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This protocol is to obtain cDNA from rat brain tissue micropunches for downstream qPCR. Briefly, RNA will be extracted from brain tissue and will be converted to cDNA through reverse transcription reaction. Protocols have been adapted from Zymo (https://files.zymoresearch.com/protocols/_r2060_r2061_r2062_r2063_direct-zol_rna_microprep.pdf) and New England Biolabs (https://www.neb.com/protocols/2013/01/23/first-strand-cdna-synthesis-protocols-e6560).

PROTOCOL CITATION

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28325

MATERIALS

NAME	CATALOG #	VENDOR
ProtoScript II First Strand cDNA Synthesis Kit - 150 rxns	E6560L	New England Biolabs
Molecular Biology Grade Water	10154604	Fisher Scientific
Ethanol (95 - 100%), molecular grade		
TRIZOL reagent	15596-026	Invitrogen - Thermo Fisher
Microcentrifuge tubes (1.5 ml)	C-2170	Denville Scientific Inc.
Direct-zol RNA Microprep Kit	R2062	Zymo Research

STEPS MATERIALS

NAME	CATALOG #	VENDOR
TRIZOL reagent	15596-026	Invitrogen - Thermo Fisher
Ethanol (95 - 100%), molecular grade		
Molecular Biology Grade Water	10154604	Fisher Scientific

MATERIALS TEXT

Ensure all reagents are nuclease-free (not just sterile)

EQUIPMENT

NAME	CATALOG #	VENDOR
C1000 Touch Thermal Cycler	1851148	BioRad Sciences
Nanodrop 2000C	TSC-ND2000C	Thermo Scientific

SAFETY WARNINGS

Wear lab coat, gloves, and goggles as the protocol involves use of Trizol. Change gloves frequently to avoid contamination.

BEFORE STARTING

Make sure all surfaces/tools/equipment are sterile and/or have been cleaned with RNase decontaminant spray. Make sure centrifuge has been pre-cooled to 4C. Thaw tissue samples on ice right before starting protocol.

1 Remove tissue samples from **-80 °C** and thaw on ice

2 If samples are stored in RNALater ICE, remove from tube and add **300 µl**

TRIZOL reagent
by Invitrogen - Thermo Fisher
Catalog #: 15596-026

to each sample. Pipet homogenize until tissue is fully dissolved and leave at RT while working on other samples. Spin samples at 13000 RPM at **4 °C** for **00:05:00** and transfer supernatant to new tube.

If samples were stored directly in Trizol, ensure tissue is fully dissolved in Trizol, and spin down as directed.

3 Add **300 µl**

Ethanol (95 - 100%), molecular grade

to each sample, mix well (invert tube, do not pipet), and transfer mixture to spin columns (with collection tube underneath). Spin samples at 13000 RPM at room temperature (**21 °C**) for **00:00:30** and transfer column to new collection tube.

4 Add **400 µl** Direct-zol RNA Prewash buffer to each column. Spin samples at 13000 RPM at room temp (**21 °C**) for **00:00:30** and discard flowthrough. Repeat this step (2 total washes).

5

Add **700 µl** RNA Wash buffer to each column. Spin at 13000 RPM at room temp (**21 °C**) for **00:02:00** . Spin one more time for **00:01:00** to ensure all liquid is removed from column.

6

Add **8 µl**

Molecular Biology Grade Water
by Fisher Scientific
Catalog #: 10154604

directly to column matrix (be careful not to pierce column) and spin at 13000 RPM at room temp (**21 °C**) for **00:00:30** . IMMEDIATELY TRANSFER COLUMNS TO ICE. Quanitify RNA concentration and purity (260/280) using

Nanodrop 2000C
Thermo Scientific TSC-ND2000C

Make sure all RNA samples stay on ice and as sterile as possible to avoid RNase contamination. Move immediately to next step to limit RNA degradation.

7 On ice, in separate 200 uL PCR strip tubes, mix up to **1 µg** RNA (up to **6 µl**) with **2 µl** d(T)23VN and

Molecular Biology Grade Water
by Fisher Scientific
Catalog #: 10154604

(if needed) to make an **8 µl** total reaction. Mix samples well and make sure nothing is sticking to the sides of the tube. Make 1 additional tube to serve as negative control.

Master mix of d(T)23VN and/or H2O should be made to avoid pipetting in and out of stock tube

8 Denature RNA at **70 °C** for **00:05:00** and cool down at **4 °C** for **00:05:00** . Use preset "cDNAincubate" function on

C1000 Touch Thermal Cycler
PCR machine
BioRad 1851148

. Immediately put on ice.

9 To each sample tube, add **10 µl** M-MuLV Reaction Mix and **2 µl** M-MuLV Enzyme Mix. To the negative control tube, add **10 µl** M-MuLV Reaction Mix and **2 µl**

Molecular Biology Grade Water
by Fisher Scientific
Catalog #: 10154604

. Total reaction volume for all samples should be **20 µl** .

Ensure this step is done on ice

10 Incubate samples at **42 °C** for **01:00:00** and then inactivate enzyme at **80 °C** for **00:05:00** . Use preset "NEB cDNA" program on

C1000 Touch Thermal Cycler
PCR machine
BioRad 1851148

At this point, samples can either be used directly in qPCR assay or stored for later use at **-20 °C**