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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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https://protocols.io/view/rat-brain-tissue-rna-extraction-cdna-synthesis-for-7wdhpa6

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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)

EOUIPMENT

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

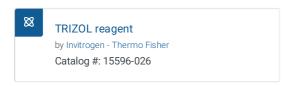
SAFETY WARNINGS

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**



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 8 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

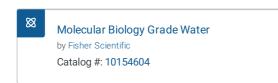
to each sample, mix well (invert tube, do not pipet), and transfer mixture to spin columns (with collection tube underneath). Spin samples at 13000 RPM at room temperature (§ 21 °C) for © 00:00:30 and transfer column to new collection tube.

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).



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.

Add **38 μl**



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



- 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.
- On ice, in separate 200 uL PCR strip tubes, mix up to 11 µg RNA (up to 16 µl) with 12 µl d(T)23VN and
 - Molecular Biology Grade Water by Fisher Scientific Catalog #: 10154604

(if needed) to make an 🔲 8 μ l 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
- Denature RNA at § 70 °C for © 00:05:00 and cool down at § 4 °C for © 00:05:00 . Use preset "cDNAincubate" function on



- . 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 $\;\; \mbox{\Large \square} 20 \; \mu \mbox{\Large I} \;\;$.
 - 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 8 -20 °C