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qPCR Assay

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

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ABSTRACT

This protocol is for running qPCR assays using previously generated cDNA. This protocol has been adapted from ThermoFisher (https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2Fcms_042179.pdf&title=UHJvdG9jb2w6IFBvd2VyIFNZQllgR3JlZW4gUENSIE1hc3RlciBNaXggYW5kIFJULVBDUg==)

PROTOCOL CITATION

Kokila Shankar, Olivier George 2020. qPCR Assay. **protocols.io** https://protocols.io/view/qpcr-assay-7whhpb6

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Oct 02, 2019

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28329

GUIDELINES

This protocol was used for quantifying nAChR mRNA levels in rat brain tissue. Some aspects of the protocol (primer concentration, PCR cycle steps) may need to be adjusted for other primers or template DNA.

MATERIALS

NAME	CATALOG #	VENDOR
Molecular Biology Grade Water	10154604	Fisher Scientific
qPCR primers		
Power SYBR™ Green PCR Master Mix	4368577	Thermo Fisher
PCR Tubes & Caps, RNase-free, 0.2 mL (8-strip format)	AM12230	Thermo Fisher
Hard Shell 384-well Plate	#HSP3805	Bio-rad Laboratories
384-well Plate Sealing Flim	#MSB1001	Bio-rad Laboratories

EQUIPMENT

NAME	CATALOG #	VENDOR
CFX384 Touch	#1855484	Bio-rad Laboratories

BEFORE STARTING

Ensure all primers have been tested to optimize concentration and reaction temperatures and efficiency has been calculated (if performing relative quantification analysis). If performing absolute quantification analysis, ensure standard cDNA is prepared. Thaw all reagents on ice.

1 Make cDNA and H2O master mix by combining **20 ng** cDNA with enough H2O for a total of **4.5 µl** , and multiplying these values by the number of wells needed. Samples should be run in at least duplicate, but preferably triplicate.

Make sure to account for pipetting error and make slightly more master mix than needed (~5%)

2 Make SYBR and primer master mix by combining **5 µl** SYBR, **0.25 µl** **10 Micromolar (µM)** forward primer, and **0.25 µl** **10 Micromolar (µM)** reverse primer, and multiplying these values by the amount of wells needed.

Make sure to account for pipetting error and make slightly more master mix than needed (~5%)

3 Add **4.5 µl** cDNA + H2O mix and **5.5 µl** SYBR + primers mix to each well, for a total reaction volume of **10 µl** . Mix master mixes frequently while pipetting, and make sure contents of each well are mixed properly.

Having a template can be helpful to ensure everything gets pipetted correctly!

4 Seal plate thoroughly, spin down quickly (2400 rpm for **00:01:00** is enough), and run reaction at the following conditions:

1. **95 °C** for **00:10:00**
2. **95 °C** for **00:00:15**
3. **59 °C** for **00:01:00**
4. Repeat steps 2-3 39X
5. Run melt curve

Reaction is run using

CFX384 Touch
qPCR machine
BioRad #1855484