NucleoGene
GENE EXPRESSION ASSAY
Instructions for Use

Release Date— 01.12.2022

Store at -20°C

REF

For research use only.
Not suitable for diagnostic use.
For professional use only.

NGOT005 250 rxn
NGOT006 250 rxn
NGOT007 250 rxn
NGOT008 250 rxn

Shipping: Shipped at gel ice and dry ice.

Storage Conditions: Store at -20 °C

Additional Storage Conditions: Stock solutions in TE buffer should be aliquoted and stored at -20 °C

Shelf Life: 12 months

Applications:

Plasmid and genomic DNA preparation Removal of RNA from recombinant protein preparations Ribonuclease protection assays Mapping single-base mutations in DNA or RNA

Description

NucleoGene Biotechnology offers comprehensive collections of predesigned, preformulated primers or primer-probe sets that help researchers perform quantitative gene expression studies on a variety of species.

• NucleoGene Gene Expression Assays – Target protein-coding transcripts from a variety of species, including human, mouse, rat, Arabidopsis, C. elegans, and Drosophila.
•NucleoGene Gene Expression RNA Assays – Target long non-coding RNA (ncRNA) in human, mouse, and rat species. These assays are designed to ncRNAs that are >60 nt in length.

This protocol provides instructions for real-time reverse transcription-PCR (real-time RT-PCR) using NucleoGene Gene Expression Assays and TaqMan Non-coding RNA Assays. Both assays are compatible with the same instruments and master mixes, and real-time RT-PCR is performed using the same procedure. Unless explicitly stated otherwise, the term "NucleoGene Gene Expression Assays" is used throughout this guide to mean either assay type.

NucleoGene Gene Expression Assays and TaqMan Non-coding RNA Assays include:
• One tube for each assay that is ordered. The tube contains:
– Two unlabeled primers (1× final concentration is 500 nM per primer)
– One 6-FAM™ dye-labeled TaqMan® LNA probe (1× final concentration is 250 nM) or SYBR GREEN Primers

Required Materials

- Reverse transcription reagents
- PCR reagents
- Thermal cycler (or real-time PCR instrument)
- Real-time PCR instrument
- Reaction plates and accessories for your real-time PCR
- Centrifuge (with plate adapter)
- Disposable gloves
- Microcentrifuge
- Pipette tips, aerosol-resistant
- Pipettors (positive/air-displacement or multichannel)
- Polypropylene tubes (various sizes)
- Vortexer
- Nuclease-free water (no diethyl pyrocarbonate [DEPC])

Prepare the Reaction

1. Thaw on ice, completely resuspend by gently vortexing, then briefly centrifuge to bring liquid to the bottom of the tube:
· NucleoGene Gene Expression Assays (4×)
· cDNA samples
2. Mix the master mix reagent by gently swirling the bottle

Calculate the number of reactions that you need for each assay.

Nucleogene Biotechnology recommends performing three replicates of each reaction. Be sure to include on each plate:
· A NucleoGene Gene Expression Assay for each cDNA sample
· Endogenous control assays
· (Optional) No template controls (NTCs) for each gene expression assay on the plate

Prepare the PCR Reaction Mix

1. Thaw on ice, completely resuspend by gently vortexing, then briefly centrifuge to bring liquid to the bottom of the tube:
· NucleoGene Gene Expression Assays (4×)
· cDNA samples
2. Mix the master mix reagent by gently swirling the bottle

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PCR reaction mix component	Volume per 20-µL reaction (µL)	
	Single reaction	Three replicates#
4× NucleoGene Gene Expression Assay (Oligo Mix)	5	20
2× NucleoGene qPCR Probe or Sybr Green Master Mix	10	40
cDNA template (1 to 100 ng)	4	16
RNase-free water	1	4

 # Replicate volumes include 20% excess to compensate for volume loss from pipetting.

2. Cap the tube and invert it several times to mix the reaction components.
3. Centrifuge the tube briefly.

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Load the plate

1. Transfer 20 µL of PCR reaction mix into each well of a 48-, 96-, or 384-well reaction plate.
2. Seal the plate with the appropriate cover.
3. Centrifuge the plate briefly.
4. Load the plate into the instrument.

Real Time PCR Protocol

Initialdenaturation	1 cycle	95ºC 15 min
Denaturation	45 cycles	95ºC 15s
Annealing		60ºC 60s*

***Reading is taken from FAM or SYBR GREEN Channel. FAM channel is used in Primer-Probe Assays, SYBR GREEN Channel is used only in Primar Assays.**

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