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Data Sheet

IDO2 Primer Mix-qPCR Assay

Catalog: #71281

Gene Description	*Homo sapiens* indoleamine 2,3-dioxygenase 2 (IDO2), mRNA.
Gene Synonym	IDO2, INDOL1
NCBI GeneID	169355
GenBank Accession	NM_194294
Uniprot	Q6ZQW0
Species	Human
Coding DNA Length	1263
Assay Type	SYBR® Green
Amplicon Size (bp)	113
Amplicon Sequence	TTCCATTTGTCGAAGTCTCCAGGAACTTGGGGCTCCCTCCTA TCCTGGTCCACTCAGACTTGGTGCTGACGAACTGGACCAAAA AAGATCCAGACGGATTCCTGGAAATTGGG
Assay Design	Intron-spanning
Purification	Desalted
Efficiency (%)	91.1
R^2	0.9978
cDNA Tm	80.0°C
Real-Time PCR Instrument	CFX Connect Real-Time PCR Detection System
Real-Time PCR Supermix	iTaq™ Universal SYBR® Green Supermix (Bio-Rad, cat. #1725120)

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Generalized Assay Protocol

The following procedure is designed to use iTaq™ Universal SYBR® Green Supermix in a 10 µl reaction. To use the qPCR reagent in different formats, follow the manufacturer's recommended assay protocol. The concentration of cDNA sample and qPCR primers should be optimized according to the sample quality and study requirements.

All amounts and volumes in the following setup are provided on a per reaction basis.
Note: we recommend setting up each condition in at least triplicate, and preparing a reaction master mix for multiple wells.

1. Thaw iTaq™ universal SYBR® supermix and other frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solutions at the bottom of tubes, then store on ice, protected from light.

2. Prepare (on ice or at room temperature) enough assay master mix for all reaction by adding all required components, except the DNA template. For one reaction, use 5 µl SYBR® 2× supermix and 1 µl 10× IDO2 qPCR primer mix (3 µM 10× primer mix would give a final concentration of 300 nM each primer. Usually, 300 nM -500 nM final concentration of each primer is optimal for qPCR reaction.) and the variable amount of TCEP-treated DNase-free H_2O to add up to 10 µl total reaction volume including DNA template to be added in the end.
Note: Usually, 100 ng-100 fg of cDNA or 50 ng-5 pg of genomic DNA is used as the DNA template in one qPCR reaction.

3. Mix the assay master mix thoroughly to ensure homogeneity and dispense equal aliquots into each qPCR tub or into the wells of a qPCR plate. Good pipetting technique must be employed to ensure assay precision and accuracy.

4. Add DNA samples to the PCR tubes or wells containing assay master mix, seal tubes or wells with flat caps or optically transparent film, and vortex 10 seconds or more to ensure thorough mixing of the reaction components. Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottom.

5. Program the thermal cycling protocol on a qPCR instrument according the following steps: Polymerase activation and DNA denaturation at 95°C, 20-30 seconds for cDNA or 2-5 minutes for genomic DNA; 2-5 seconds of denaturation at 95°C, 15-30 seconds of annealing/extension and plate read at 60°C, repeat 2-5 seconds denaturation, annealing/extension and plate read step for 40 cycles. Perform melt curve analysis at the end by increasing temperature from 65-95°C in 0.5°C increments at 2-5 seconds.

6. Load the PCR tubes or plate into the qPCR instrument and start the PCR run.

7. Perform data analysis according to the instrument-specific instructions.

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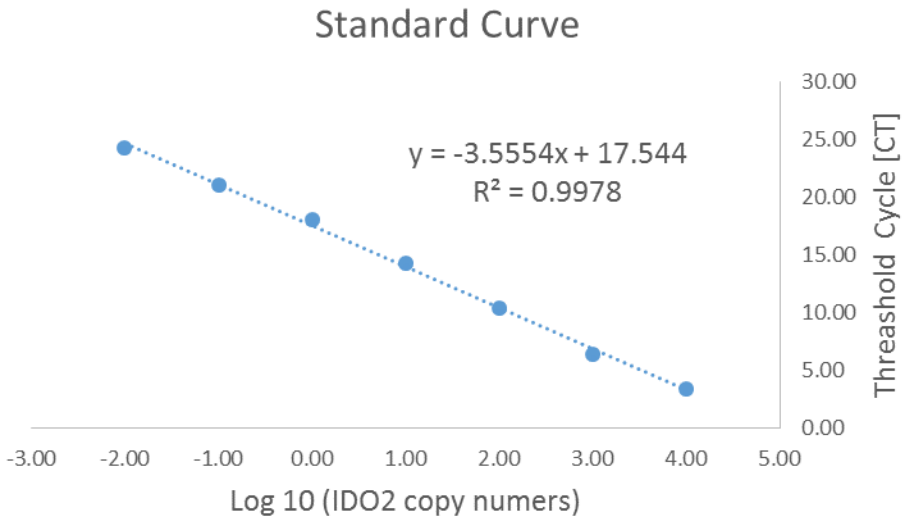

Figure 1. qPCR standard curve generated with known concentration of hIDO2 cDNA on linearized plasmid.