# Quantikine® mRNA Probes and Calibrator Kit

## Human p21/CIP1/CDKN1A

**Catalog Number:** RN1047
**Lot Number**:
**Expiration Date:**

## *Specifications and Use*

**Components**

- Probes are supplied as a 6X concentrated stock solution.
- RNA Calibrator is supplied as a 1200 attomole/mL (amol/mL) stock solution (1.1 mL).

**RNA Calibrator Sequence and Size**

- Genbank® Accession Number: NM_000389
- Cloned cDNA size: 2140 base pairs (bp)

**Other Supplies Required**

- Quantikine mRNA Base Kit (Catalog Number RN000).

**Storage**

- Store unopened kit at ≤ -70° C. Do not use past the expiration date above.
- **Avoid repeated freeze-thaw cycles.**

**Instructions for Use**

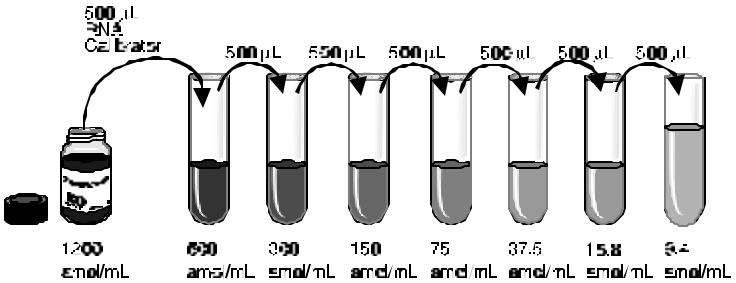
- Completely thaw reagents before use.
- Mix reagents by gentle inversion.
- Microcentrifuge briefly before use to prevent the loss of reagents.
- Refer to the Base Kit manual for the Quantikine mRNA assay procedure.

**Preparation of Probes**

- For 96 wells, add 1.0 mL of the probes to 5.0 mL of Sample Diluent (provided in the Quantikine mRNA Base Kit).
- For 36 wells, add 0.4 mL of the probes to 2.0 mL Sample Diluent.
- Freeze the remaining undiluted probes at ≤ -70° C.
- Make a fresh dilution of probes before running each assay.

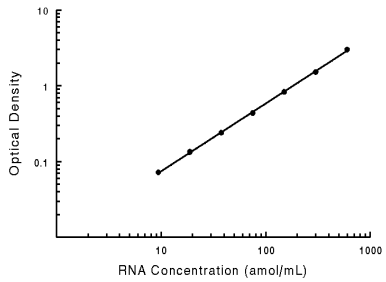
**Preparation of RNA Calibrator**

- Pipette 500 µL of Calibrator Diluent into each tube. Pipette 500 µL of RNA Calibrator into the 600 amol/mL tube. Continue the two-fold dilution series (shown below). Mix each tube thoroughly before each transfer. The 600 amol/mL calibrator serves as the high calibrator. The Calibrator Diluent serves as the zero calibrator.
- Freeze the remaining undiluted RNA Calibrator at ≤ -70° C.
- Make a fresh dilution of calibrators before running each assay.




## *Typical Data*

This human p21 calibrator curve is provided only for demonstration. A calibrator curve should be generated each time an assay is run.




| amol/mL | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.131<br>0.141 | 0.136 | - |
| 9.4 | 0.203<br>0.212 | 0.208 | 0.072 |
| 18.8 | 0.265<br>0.274 | 0.270 | 0.134 |
| 37.5 | 0.379<br>0.373 | 0.376 | 0.240 |
| 75 | 0.569<br>0.577 | 0.573 | 0.437 |
| 150 | 0.984<br>0.945 | 0.965 | 0.829 |
| 300 | 1.623<br>1.695 | 1.659 | 1.523 |
| 600 | 3.002<br>3.278 | 3.140 | 3.004 |

## *Performance Characteristics*

**Sensitivity -** Seven assays were evaluated and the minimum detectable dose (MDD) of human p21 mRNA ranged from 1.7 - 3.7 amol/mL. The mean MDD was 2.5 amol/mL. The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of 20 zero calibrator replicates and calculating the corresponding concentration.

**Intra-assay Precision -** Three samples of known concentration were assayed twenty times on one plate to assess precision within an assay.

| Sample | 1 | 2 | 3 |
|---|---|---|---|
| n | 20 | 20 | 20 |
| Mean (amol/mL) | 87.8 | 244 | 464 |
| Standard Deviation | 4.8 | 12.7 | 15.5 |
| % CV | 5.5 | 5.2 | 3.3 |

**Inter-Assay Precision -** Three samples of known concentration were assayed twenty times in separate assays to assess precision between assays.

| Sample | 1 | 2 | 3 |
|---|---|---|---|
| n | 20 | 20 | 20 |
| Mean (amol/mL) | 86.2 | 238 | 434 |
| Standard Deviation | 7.3 | 14.4 | 28.0 |
| % CV | 8.5 | 6.1 | 6.5 |

**Sample Data -** MCF-7 cells were treated with 20 Gray γ-irradiation, then allowed to recover for 4 hours. Poly $(A)^+$ RNA was isolated using an Oligotex® mRNA Kit (Qiagen, Inc.).

| Sample | Sample Amount (ng) | Expected amol/mL | Observed amol/mL |
|---|---|---|---|
| Irradiated MCF-7 cells | 500 | - | 438 |
| | 250 | 219 | 216 |
| | 125 | 109 | 109 |
| | 62.5 | 54.7 | 56.4 |
| | 31.3 | 27.3 | 26.9 |
| | 15.6 | 13.7 | 13.1 |

## *Technical Hints:*

- The amount of RNA needed per well is dependent on the sample type. For initial analysis, $5 \times 10^5$ to $2 \times 10^6$ cells/mL of Cell Lysis Diluent is recommended for cell lysate samples and 2 - 5 µg of total RNA per well is recommended for total RNA samples. Poly $(A)^+$ RNA samples may require less RNA than total RNA samples. The amount required for subsequent analyses can be adjusted after the target mRNA concentration is known. In sample types tested at R&D Systems, results were obtained using 250 - 5000 ng of total RNA and 15.6 - 500 ng of poly $(A)^+$ RNA.
- The approximate molecular weight for single stranded RNA (ssRNA) can be calculated by multiplying its length in nucleotides times the average mass per mole of RNA nucleotide (320 g/mol nucleotide).
- One attomole of human p21 mRNA is approximately 685 femtograms ($1 \text{ g} = 10^{15}$ fg). This conversion factor uses an estimated molecular weight for the ssRNA that is based on the cDNA size obtained from Genbank. The cDNA size does not include the cap structure or the poly (A) tail and may not include the entire untranslated regions.
- Expression of human p21 mRNA is tightly regulated and elevated levels may be transient.
- Human p21 mRNA may not be detectable in unstimulated cells or in stimulated cells that were harvested when the mRNA was not near peak levels.



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