

MegaPlex™ Primer Pools

Catalog Numbers 4399966, 4399970, 4399233, 4399203, 4444281, 4444303, 4444292, and 4444308

Pub. No. 4401697 Rev. E

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Applied Biosystems™ MegaPlex™ RT Primers greatly streamline the workflow for quantitating up to 381 microRNAs in parallel by enabling reverse transcription of cDNA for all microRNAs in a single reaction; they are available for human and rodent (mouse and rat) species. A companion pool of Applied Biosystems™ MegaPlex™ PreAmp Primers enables an optional preamplification step to be added to the workflow in situations where sample is limiting, or sensitivity is of the utmost importance. For comprehensive coverage, two pools each (pool A and pool B) of MegaPlex™ RT Primers and MegaPlex™ PreAmp Primers are available for human and rodent species, respectively. The MegaPlex™ solution can be run either with individual TaqMan™ MicroRNA Assays or, for the ideal profiling workflow, with matched TaqMan™ Array MicroRNA Cards.

Contents and storage

| Cat. No. | Primer pool | Contents | Storage |
|---------------------------------|--------------------|--|--|
| MegaPlex™ RT Primers | | | |
| 4399966 | Human Pool A v2.1 | <ul style="list-style-type: none"> RT primers (1 tube) MgCl₂ (1 tube) 50 reactions | Ships at ambient temperatures. Store the MegaPlex™ RT primer pools at -25°C to -15°C. |
| 4444281 | Human Pool B v3.0 | | |
| 4399970 | Rodent Pool A v2.0 | | |
| 4444292 | Rodent Pool B v3.0 | | |
| MegaPlex™ PreAmp Primers | | | |
| 4399233 | Human Pool A v2.1 | PreAmp primers (1 tube; 50 reactions) | Ships at ambient temperatures. Store the MegaPlex™ PreAmp primer pools at -25°C to -15°C. |
| 4444303 | Human Pool B v3.0 | | |
| 4399203 | Rodent Pool A v2.0 | | |
| 4444308 | Rodent Pool B v3.0 | | |

Workflow

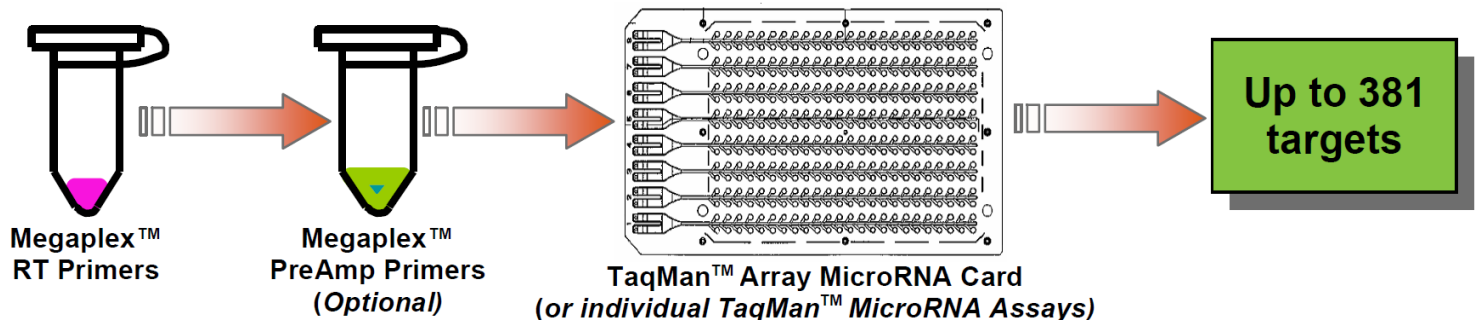


Fig. 1 Workflow using MegaPlex™ primer pools and TaqMan™ Array MicroRNA Card

Data normalization

Each TaqMan™ Array Card of the two-card set (either human or rodent) contains identical candidate endogenous control assays that provide a variety of options for normalization.

| Endogeneous control | Species | Comments |
|---------------------|-----------------------|--|
| Mammalian U6 | Human, mouse, and rat | Repeated 4x on card enabling monitoring of assay reproducibility |
| RNU48 | Human | Small nucleolar RNA |
| RNU44 | Human | Small nucleolar RNA |
| snoRNA135 | Mouse | Small nucleolar RNA |
| snoRNA202 | Mouse | Small nucleolar RNA |
| U87 | Rat | Small nucleolar RNA |
| Y1 | Rat | Small non-coding RNA |
| ath-miR159a | <i>Arabidopsis</i> | Not detectable in mammalian species For use as a negative or processing control |

For more information on data normalization, see the Application Note *Endogenous Controls for Real-Time Quantitation of miRNA Using TaqMan™ MicroRNA Assays* (Pub. No. 127AP11-01).

MegaPlex™ assay performance

MegaPlex™ RT and PreAmp Primers are highly multiplexed pools designed to significantly streamline the workflow when profiling many miRNA targets in a single experiment. When combined in a large multiplex pool, a small subset of assays exhibit lower no-template-control (NTC C_T values < 35) values than when run individually. This is not an assay design issue. In the interest of providing the broadest coverage possible, customers have requested that we include these assays on the profiling array, but with the expectation that they are to be considered semi-quantitative. As a result, fold-change measurements using these assays may be less than

the true value. To aid data interpretation, the identity of these assays is provided in the MegaPlex™ Assay Performance File. If accurate fold-change measurements for these assays is required, we recommend that changes detected using the MegaPlex™ assay workflow be validated using the corresponding individual TaqMan™ MicroRNA Assay.

MegaPlex™ Assay Performance File

The MegaPlex™ Assay Performance File is provided on the CD accompanying the TaqMan™ Array MicroRNA Cards.

| File | Cat. No. |
|---|----------|
| MEGAPLEX ASSAY PERFORMANCE FILE_HUMAN A v2.1 | 4403210 |
| MEGAPLEX ASSAY PERFORMANCE FILE_HUMAN B v3.0 | 4428133 |
| MEGAPLEX ASSAY PERFORMANCE FILE_RODENT A v2.0 | 4403212 |
| MEGAPLEX ASSAY PERFORMANCE FILE_RODENT B v3.0 | 4455463 |

Documentation

Supporting documentation is included on the CD accompanying the TaqMan™ Array MicroRNA Cards, and is available at thermofisher.com.

| Publication | Pub. No. |
|--|----------|
| <i>MegaPlex™ Pools for microRNA Expression Analysis Protocol</i> | 4399721 |
| <i>MegaPlex™ Pools Quick Reference Card</i> | 4399813 |

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Table 1 Revision history of Pub. No. 4401697

| Revision | Date | Description |
|----------|---------------|------------------------------------|
| E | 21 March 2016 | Format, style, and legal updates |
| D | 09 June 2010 | Baseline for this revision history |

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Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

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