

RT² Nano PreAMP cDNA Synthesis Primer Mix – Human Signal Transduction PathwayFinder™

Catalog Number
PBH-3014

Description
For 12 Samples

Description

The RT² Nano PreAMP cDNA Synthesis Primer Mix is used for pre-amplification of cDNA from nanogram amounts of RNA (1-100ng) for multi-gene expression analysis with RT²Profiler Human Signal Transduction PathwayFinder™ PCR Arrays. The RT² Nano PreAMP cDNA Human Signal Transduction PathwayFinder™ Primer Mix, together with the RT² Nano PreAMP cDNA Synthesis Kit, utilizes multiplex PCR-based pre-amplification to provide amplification of 89 gene-specific cDNA target templates with minimal bias for analysis with the RT²Profiler Human Signal Transduction PathwayFinder™ PCR Arrays.

Contents

Please check the kit components immediately after you receive this package. We are not responsible for any missing items not reported within two (2) business days upon receipt.

- PBH-3014:
- One (1) tube containing 90µl RT² Nano PreAMP cDNA Human Signal Transduction PathwayFinder™ Primer Mix solution
 - Enough for 12 25-µl standard Nano PreAMP reactions

Note: The performance of RT² Nano PreAMP cDNA Synthesis Human Signal Transduction PathwayFinder™ Primer Mix is only guaranteed when it is used in conjunction of RT² Nano PreAMP cDNA Synthesis Kit and RT²Profiler Human Signal Transduction PathwayFinder™ PCR Arrays. No other PCR Arrays should be used to analyze the pre-amplified samples.

Important Note:

- 1. Please verify that the lot number of this RT² Nano PreAMP cDNA Synthesis primer mix is compatible with the lot number(s) of the RT²Profiler PCR Array listed on the attached sheet before use. Otherwise the Nano PreAMP process may not work optimally.**
2. For future PCR Array purchase to use with this lot of primer mix, please check with our Customer service (1-888-503-3187) before ordering to ensure the available PCR Arrays are compatible.

Storage Conditions

The product is shipped frozen or at ambient temperature. Store at -20 °C immediately upon receipt. If entire volume is not be used all at once, divide into aliquots and store at -20 °C. Avoid repeated freezing and thawing.

Shelf Life

All reagents are stable for 6 months after receipt if stored at the recommended temperature.

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Product Specification Sheet

Brief Protocol

Read carefully the User Manual of the RT² Nano PreAMP cDNA Synthesis Kit prior to the first-time use. Refer to the instructions in the User Manual every time you use the RT² Nano PreAMP cDNA Synthesis Kit (catalog # C-06).

1. Ensure that you do not contaminate the RT² Nano PreAMP cDNA Synthesis Primer Mix by using a **fresh** pipette tip every time you draw an aliquot from the tube.
2. Perform first strand synthesis use RT² Nano PreAMP cDNA Synthesis Kit as follows:

Add 2 µl of GE to 8 µl of RNA (1-100ng). Incubate at 42°C for 5 min and immediately chill on ice.
Mix a master mix for the RT reaction as below:

	For 1 reaction:
BC3	4 µl
RE	1 µl
RI	1 µl
P2	1 µl
RNase-free H ₂ O	3 µl

Add 10 µl of the RT master mix to 10 µl GE-treated RNA.
Incubate at 42°C for 30 min and heat at 95°C for 5 min. Chill on ice or store at -20°C until use.

3. For the normal standard reaction, mix the following components in a PCR tube:

12.5 µl	2X RT ² PreAMP PCR Master Mix (PA-030)
7.5 µl	RT ² Nano PreAMP cDNA Synthesis Primer Mix for the RT ² Profiler™ PCR Array of your choice
5.0 µl	<u>template undiluted cDNA from a 20-µl first strand synthesis reaction</u>
25.0 µl	final volume
4. Perform 12 cycles of PCR in a thermal cycler:
NOTE: *The 10 min step at 95 °C is required to activate the HotStart Taq DNA polymerase.*
95 °C, 10 min; 12 cycles of (95 °C, 15 sec; and 60 °C, 2 min); 4 °C forever
5. Add 2 µl of the Side Reaction Reducer (SR1) to each pre-amplified reaction, and incubate at 37 °C for 15 min followed by heat inactivation at 95 °C for 5 min.
6. Dilute the 27-µl pre-amplified templates to 111 µl by adding 84 µl of dd H₂O. Use immediately and keep on ice prior to loading onto the RT²Profiler™ PCR Array or store at -20°C until use.
7. For use in the RT²Profiler™ PCR Array, mix well the following components in 15-mL conical tube:

1275 µl	2X SABiosciences RT ² qPCR SYBR Green Master Mix (Note: Use the appropriate master mix specific for your real-time PCR instrument)
102 µl	diluted PreAMP PCR reaction (Step 6)
1173 µl	<u>ddH₂O</u>
2550 µl	final volume
8. Add 25 µl of the above Experimental Cocktail to each well of the PCR Array, preferably from a reservoir with an eight-channel pipettor (or a twelve-channel pipettor but only using eight tips).
9. Run the following real-time thermal cycler program:
NOTE: *The 10 min step at 95 °C is required to activate the HotStart Taq DNA polymerase.*
95 °C, 10 min; 40 cycles of (95 °C, 15 sec; and 60 °C, 60 sec)
10. Program the real-time thermal cycler to detect and record the SYBR® Green I signal from every reaction during the annealing step of each cycle.