Random Primers

Catalog Number: RDPC2
Pack Size: 80 tests

Specifications and Use

Description
- Random primers (N_6) are comprised of hexamers that contain arbitrary deoxyribonucleotides at each of the six positions. These primers anneal to complementary sequences on target DNA or RNA and serve as primers for DNA synthesis by a DNA polymerase or reverse transcriptase.

Components
- **Random Primers** - Lyophilized. Each vial contains 25 µg. Adjust to a final concentration of 300 ng/µL by resuspending primers in 83 µL nuclease-free water.

Storage
- Random primers resuspended in nuclease-free water are stable for up to one year at ≤ -20° C in a non-frost free freezer. Aliquot in single use portions. **Do not use past the expiration date above.**
- **Avoid repeated freeze-thaw cycles.**

We recommend the following protocol for R&D Systems' Random Primers.

Reverse Transcription Reaction:

1. Thaw all reagents completely on ice. All reactions should be assembled on ice.
2. Resuspend the RT primers according to the instructions in the "Specifications and Use" section.
3. Pipet the following into a nuclease-free tube:
   1 to 5 µg of total RNA (up to 11 µL)
   1 µL random primers (300 ng/µL)
   x µL Nuclease-free dH_2O for a final volume of 12 µL
4. Mix and incubate at 70° C for 10 minutes. Place tube on ice immediately.
5. Briefly centrifuge the tube, then add the following to each tube:
   4 µL 5X Reverse Transcription Buffer
   2 µL 0.1M DTT
   1 µL 10 mM dNTPs
6. Mix and incubate at room temperature (~25° C) for 10 minutes.
7. Incubate at 42° C for 2 minutes.
8. Add 1 µL RNase H^- Reverse Transcriptase (200 units/µL). Mix by pipetting.
9. Incubate at 42° C for 50 minutes.
10. Incubate at 70° C for 15 minutes.
11. Dilute reactions 5-fold by adding 80 µL of nuclease-free dH_2O.
12. Store at ≤ -20° C in a non-frost free freezer.

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