mRNA Fragmentation Protocol (E6100)

Overview

Starting Material: Purified mRNA (50–250 ng)

Protocol

1. **mRNA Fragmentation Protocol**
    1. Mix the following components in a sterile PCR tube:
       Purified mRNA: 1–18 µl
       10X RNA Fragmentation Reaction Buffer: 2 µl
       Nuclease-Free Water: variable

       Total volume: 20 µl
    2. Incubate in a preheated thermal cycler for 5 minutes at 94°C. This is the optimal condition for eukaryotic mRNA (see Figure 1 on the product page). Other types of mRNA may require optimizing incubation time to obtain desired fragment size distribution.
    3. Transfer tube to ice.
    4. Add 2 µl 10X RNA Fragmentation Stop Solution.
2. **Clean Up Fragmented RNA Using RNeasy MinElute Spin Columns**
    1. Add 78 µl of the Nuclease-Free Water to the 22 µl fragmented RNA from step 4. Purify sample using RNeasy MinElute Cleanup Kit (Qiagen #74204) following manufacture instructions. Elute in 15.5 µl Nuclease-Free Water. The recovered volume should be ~14.5 µl.

       Note: column purification removes short RNA Fragments and enriches the sample for RNA fragments longer than 200 nucleotides.
3. **Alternatively, Clean Up Fragmented RNA Using Ethanol Precipitation**
    1. Mix the following components in a sterile 1.5 ml microcentrifuge tube:
       Fragmented RNA from Step 4: 22 µl
       3M Sodium Acetate, pH 5.2: 2 µl
       Linear Acrylamide, 10 mg/ml: 1–2 µl
       100% Ethanol: 60 µl

       Total volume: 85–86 µl
    2. Incubate at -80°C for 30 minutes.
    3. Centrifuge at 14,000 rpm for 25 minutes at 4°C in a microcentrifuge.
    4. Carefully remove ethanol.
    5. Wash pellet with 300 µl of 70% ethanol.
    6. Centrifuge and carefully remove 70% ethanol.
    7. Air dry pellet for up to 10 minutes at room temperature to remove residual ethanol.
    8. Resuspend in 14.5 µl Nuclease-Free Water.