

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 μ l

10X RNA Fragmentation Reaction Buffer: 2 μ l

Nuclease-Free Water: variable

Total volume: 20 μ l

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 μ l 10X RNA Fragmentation Stop Solution.

2. Clean Up Fragmented RNA Using RNeasy MinElute Spin Columns

1. Add 78 μ l of the Nuclease-Free Water to the 22 μ l fragmented RNA from step 4. Purify sample using RNeasy MinElute Cleanup Kit (Qiagen #74204) following manufacture instructions. Elute in 15.5 μ l Nuclease-Free Water. The recovered volume should be ~14.5 μ l.

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 μ l

3M Sodium Acetate, pH 5.2: 2 μ l

Linear Acrylamide, 10 mg/ml: 1–2 μ l

100% Ethanol: 60 μ l

Total volume: 85–86 μ l

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 μ l 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 μ l Nuclease-Free Water.