HYBRID
LAB NETWORK

In vitro gene editing - RNA purification

Purification of sgRNA with Quick-RNA™ Microprep Kit – 10 min

Purification of sgRNA - Needed Materials:

- Quick-RNA™ Microprep Kit (https://www.zymoresearch.com/products/quick-rna-microprep-kit);
- Nuclease-free H_2O (i.e., DNase- and RNase- free water);
- 100% ethanol;
- RNase-free tubes and filter pipette tips;
- Micropipettes.

Purification of sgRNA - Protocol:

a) Add an equal volume of **RNA Lysis Buffer** to the sample (50µL of buffer per 50µL of sample) and mix.

b) Add two volumes of 100% ethanol (200µL of ethanol per 50µL of initial sample) and mix.

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c) Transfer the mixture to the **Spin Column** in a **Collection Tube** and centrifuge for 1 min at 13 000 x g. Discard flow-through;

d) Add 400µL of **RNA Prep Buffer** to the column and centrifuge for 1 min at 13 000 x g. Discard flow-through.

e) Add 700µL of **RNA Wash Buffer** to the column and centrifuge for 1 min at 13 000 x g. Discard flow-through.

f) Add 400µL of **RNA Wash Buffer** to the column and centrifuge at 13 000 x g for 2 min to ensure complete removal of the wash buffer. Carefully, transfer the column into a nuclease-free tube.

g) Add 20µL of Nuclease-free H_2O directly to the column matrix and centrifuge for 1 min at 13 000 x g. Use immediately, or store RNA at -80°C.

h) Analyze purified sgRNA by gel electrophoresis.