Quick-Start Protocol March 2016

Oligotex® Direct mRNA Kit for animal tissues

The Oligotex Direct mRNA Kit (cat. nos. 72022 and 72041) can be stored at room temperature (15–25°C) for up to 1 year if not otherwise stated on label.

Further information

- *Oligotex Handbook*: **www.qiagen.com/HB-1287**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- For more information, including the purification of Poly A$^+$ mRNA from total RNA, and general handling advice, refer to the handbook.
- Prepare buffers and incubate according to instructions in the handbook.
- Fresh, frozen or stabilized tissue can be used.
- Unless otherwise indicated, all protocol steps, including centrifugation, should be performed at 20–30°C.
- Unless otherwise indicated, all centrifugation steps should be performed in a microcentrifuge at maximum speed (14,000–18,000 x *g*).
- ■ denotes volumes for ≤100 mg tissue; ▲ denotes volumes for 100–250 mg tissue.

1. Prepare, disrupt and homogenize tissue samples according instructions in the handbook.
2. Add Buffer ODB (see table below) to the lysate, and mix. Centrifuge for 3 min at 14,000–18,000 x *g* (microcentrifuge tubes) or 10 min at 10,000 x *g* (larger tubes). Transfer supernatant to a new RNase-free tube.
3. Add Oligotex Suspension (see table below). Mix thoroughly and place at 20–30°C for 10 min.
4. Pellet by centrifuging 5 min at 14,000–18,000 x *g* (microcentrifuge tubes) or 10 min at 10,000 x *g* (larger tubes). Carefully remove the supernatant.

Table 1. Reagent volumes

Amount of tissue, mg	Buffer OCL, ml	Buffer OCD, ml	Oligotex Suspension, µl
■ ≤10	0.6	1.2	20
■ 10–25	0.6	1.2	35
■ 25–50	0.6	1.2	70
■ 50–100	0.6	1.2	110
▲ 100–150	1.0	2.0	130
▲ 150–250	2.0	4.0	165

5. Resuspend the pellet thoroughly in ■ 100 µl or ▲ 200 µl Buffer OL1.
6. Add ■ 400 µl or ▲ 800 µl Buffer ODB, incubate at 70°C for 3 min and then place at room temperature (15–25°C) for 10 min.
7. Centrifuge for 5 min and carefully remove the supernatant.
8. Resuspend the pellet in ■ 350 µl or ▲ 600 µl Buffer OW1.
9. Pipet the sample onto a ■ small spin column or ▲ large spin column placed in a ■ 1.5 ml or ▲ 2 ml microcentrifuge tube. Centrifuge for 1 min at maximum speed. Discard the flow-through.
10. Transfer the spin column to a new RNase-free ■ 1.5 ml or ▲ 2 ml tube. Pipet ■ 350 µl or ▲ 600 µl Buffer OW2 onto the column. Centrifuge for 1 min at maximum speed, and discard the flow-through.
11. Repeat step 10 once, using the same microcentrifuge tube.
12. Transfer the spin column to a new RNase-free ■ 1.5 ml or ▲ 2 ml tube. Pipet ■ 20–100 µl or ▲ 50–300 µl hot (70°C) Buffer OEB onto the column. Resuspend the resin, and centrifuge for 1 min at maximum speed.
13. Pipet another ■ 20–100 µl or ▲ 50–300 µl hot (70°C) Buffer OEB onto the column. Resuspend the resin. Centrifuge for 1 min at maximum speed.

Scan QR code for handbook.