

BILATEST® isodirect mRNA Kit

50 Extractions

Included reagents

Reagent	Volume
(1) Suspension Buffer	30 ml
(2) Lysis Buffer	50 ml
(3) Magnetic Beads	2 ml
(4) Wash Buffer A	45 ml
(5) Wash Buffer B	90 ml
(6) Elution Buffer	5 ml

This kit contains enough materials for 50 isolations of mRNA directly from 10^7 cells, 100 mg animal tissue or 100 mg plant tissue and is optimized for use with BILATEC magnetic separators (e.g. BILATEST® Magnetic Separator M12+12 for 1.5 ml tubes, Order-No. 210141).

Storage Conditions

All solutions may be stored at room temperature (15-25°C), with the exception of **Magnetic Beads (3)** and **Lysis Buffer (2)**, which are best stored at 4°C.

All buffers should be brought to room temperature before you start.

The kit buffers contain irritant substances. Take appropriate laboratory safety measures and wear gloves and safety glasses when handling.

Samples and Protocol Adjustments

The included protocol can be individually adapted and is scalable for mRNA isolation from $10^3 - 10^7$ cells, 10 – 100 mg animal or plant tissue. 40 µl of Magnetic Bead (3) suspension can bind at least 2 µg mRNA.

UV Measurements

In some cases there may be some traces of the magnetic beads left in the eluate after removal from the tube. Such particles will not interfere with most downstream applications such as PCR but may increase the background in UV measurements. In such a case, prior to UV analysis, we recommend an additional application of the magnet to the eluate for 3 minutes in order to separate any traces of particles. For Pure mRNA the expected A_{260}/A_{280} ratio is between 1,9 – 2,3. The A_{260} value should be between 0,1 and 1,0 for significant readings.

Before you start

Ensure that the material getting in contact with RNA is free of contamination RNases.

The extreme instability of RNA is mainly due to the ubiquitous presence of enzymes (RNases) which degrade RNA, so decontaminate all equipment following protocols to create a Ribonuclease-Free Environment.

Prewash

1. Shake bead suspension vigorously and transfer 40 µl (1 mg) to a new 1,5 ml microcentrifuge tube.
2. Place the tube in a magnetic rack and separate the beads magnetically.
3. Remove the storage buffer and wash the beads by resuspending in 300 µl **Suspension Buffer (1)**.
4. Repeat step 3.
5. Resuspend the **Magnetic Beads (3)** in 100 µl **Lysis Buffer (2)**.

Preparation of Samples

Disrupt and homogenise 1 – 100 mg tissue (fresh or frozen) or 10^7 cells using one of the following methods:

1. **Bead-mill or rotor-stator**
Add 400 µl **Lysis Buffer (2)** to the sample in a 2 ml screw cap tube and homogenise according to the instrument supplier's instructions.
2. **Mortar and Pestle**
Thoroughly grind the sample in liquid nitrogen to obtain a fine powder.
Add 900 µl **Lysis Buffer (2)** to the still frozen powder in the tube.
Reduce the viscosity of the lysate using a syringe with a 21G needle (pass the liquid 10 times through the needle).

Purification Protocol for mRNA from 10³-10⁷ cells, 10-100 mg animal or plant tissue

1. Centrifuge the lysate at 13 000 rpm for 5-10 minutes to pellet the cell debris.
2. Transfer the supernatant to the tube with the washed **Magnetic Beads (3)**.
3. Resuspend the beads in the lysate and incubate for 2 – 5 minutes at 65°C.
4. Incubate for 5 – 10 minutes at room temperature with occasional gentle mixing.
5. Following incubation, place the tube in a BILATEST[®] Magnetic Separator and wait until all the beads have been attracted to the side of the tube. Aspirate off all of the supernatant and remove the tube from the magnet.
6. Add 900 µl **Wash Buffer A (4)** to the tube. Gently resuspend and wash the beads with 5 pipetting strokes.
7. Separate the beads magnetically and remove the supernatant.
8. Repeat steps 5 and 6 another 2 times with **Wash Buffer B (5)** (900 µl).
9. Add 50 – 100 µl **Elution Buffer (6)** and resuspend the beads. Incubate the suspension for 2 minutes at 70°C with vigorous mixing to facilitate complete mRNA elution.

If a RT-PCR is planned, it is possible to use 1/50 to 1/10 of the bead suspension to perform the first-strand DNA synthesis directly.

10. After incubation place the tube in the Magnetic Separator and wait until the supernatant is clear (at least 2 minutes). Transfer the eluate containing the purified mRNA to a clean RNase free tube.

To increase yield repeat steps 9 and 10.

Regeneration for Reuse

After mRNA isolation the **Magnetic Beads (3)** can be reused, if regenerated as follows:

1. Add 500 µl of 0,1 N NaOH, resuspend and incubate for 10 minutes at room temperature. Magnetically separate and discard supernatant.
2. Repeat step 1.
3. Resuspend in 500 µl of water and incubate for 5 minutes at 95°C. Separate magnetically and discard supernatant.
4. Wash twice with 500 µl TE buffer (pH 7 – 7,5).
5. For re-use start with step 2 of the isolation protocol.

Troubleshooting

Problem	Possible Cause	Recommendation/Solution
Low yield	Sample condition	<ul style="list-style-type: none"> • Sample must be placed on ice when handling.
	Insufficient Lysis or Binding to Magnetic Beads	<ul style="list-style-type: none"> • Mix samples thoroughly upon addition of lysis buffer. • It may help to extend the lysis time. • It may help to lengthen the hybridization time.
	Incomplete Elution	<ul style="list-style-type: none"> • Verify that the elution temperature was correct and, if necessary, extend the elution time.
	Wash Buffer not removed sufficiently	<ul style="list-style-type: none"> • Ensure that as much buffer as possible is removed between washing steps before proceeding further
	Bead pellet not properly resuspended in elution step	<ul style="list-style-type: none"> • Resuspend bead pellet in elution buffer until the pellet is homogeneously dispersed.
A₂₆₀ /A₂₈₀ ratio is too low	Protein contamination	<ul style="list-style-type: none"> • Beads not sufficiently resuspended during washing steps. • If necessary, repeat purification protocol omitting the lysis buffer step.
	Residual beads in eluate	<ul style="list-style-type: none"> • Incomplete separation of the Magnetic Beads from the eluate can increase the background of UV measurements. • Repeat magnetic separation and transfer eluate to a clean tube. • Residual Magnetic Beads will not affect most downstream processes.
Precipitate in reagent bottle	Bottles stored below room temperature.	<ul style="list-style-type: none"> • Warm reagent bottle in water bath to redissolve precipitate.
Degraded RNA	Old Sample, or sample has been repeatedly frozen and thawed	<ul style="list-style-type: none"> • To reduce RNase activity in frozen samples, thaw them quickly in a 37°C water bath and then place on ice until use.
	RNase contamination	<ul style="list-style-type: none"> • Replace all buffers.