TruRNA MiniKit

Patent Pending
includes reagents for 25 extractions.

Meaningful RNA Results
Co-Extraction of mRNA and DNA

Atom Sciences, Inc
www.atom-sci.com
(865) 483-1113
info@atom-sci.com

Note: Keep spin filters with resin refrigerated until use

The purpose of the ***TruRNA*** reagent system is to enable recovery of meaningful mRNA with simultaneous recovery of DNA from a wide variety of samples. It is particularly difficult to evaluate the expressed gene content of environmental samples because typical half-lives of prokaryotic mRNA are measured in seconds to minutes. These samples must be frozen in liquid N_2 immediately upon sample acquisition and maintained in cryo-storage until the nucleic acids are extracted. Extraction methods that require the sample to be brought into solution prior to cell disruption allow a change in the expressed mRNA levels in the sample. Therefore, the ***TruRNA*** system is designed so that environmental samples do not have to be thawed or brought into suspension prior to cell disruption and inactivation of cellular machinery.

The ***TruRNA*** *MiniKit* is appropriate for samples with active large microbial community populations such as A-horizon soils (except during a drought) and shallow or warm water marine sediments. Use our *Mid-Scale* ***TruRNA*** kit for other types of samples.

Items Supplied in the *TruRNA* Kit

•	Solution A- 1.3 ml/each (2)	•	Nuclease-free ddH_2O – 20 ml
•	Solution B – 40 ml	•	Sand tubes (25)
•	Solution C – 11 ml	•	TruRNA Spin Filters (25)
•	Solution D – 6 ml	•	2-ml centrifuge tubes – 100

Items Required But Not Supplied In The *TruRNA* Kit

——— **Important! Follow all safety precautions in the MSDS for each reagent!** ———

- Mortar / Pestle (appropriate size for sample)
- Liquid N_2 and a handheld liquid N_2 dewar.
- Pipettors (20, 100, 1000 µL sizes), Balance, Spatula, Gloves
- Microcentrifuge (minimum range of 1500 to 16,000 × g).
- 2-mercaptoethanol - 9.2 µl (4.6 µl for each bottle of Solution A)
- Chloroform:isoamyl alcohol 49:1 (v/v; reagent grade) – 2 ml per sample.
- Isopropyl alcohol (reagent grade) – approximately 2 ml per sample.
- Reagent system for fractionation of total nucleic acids.

Before Starting

Timing is essential to successful recovery of soil mRNA. Perform the following steps before beginning work with the sample:

- Environmental samples should be frozen in liquid N_2 and remain frozen at -80°C or colder.
- Be sure to preheat **Solution C** to 50°C before starting the *Extraction Procedure*.
- Add 4.6 µl of 2-mercaptoethanol to solution A (initial volume = 1.3 ml) mark the label to indicate that the 2-mercaptoethanol has been added.
- Verify that all equipment used for nucleic acid preparation is nuclease-free and remains in this condition throughout the experiment.

Use subject to Terms & Conditions included with kit
and available at www.atom-sci.com/TruRNA.htm

TruRNA MiniKit EXTRACTION PROCEDURE

1. Weigh out approximately 250 mg (± 10%) of frozen sample. Record the actual sample weight. Add the sample and the contents of a ***SAND*** tube into a mortar. Retain the empty sand tube for later use.
2. Add 100 µl of ***SOLUTION A*** (make sure 2-mercaptoethanol has been added as directed in the *Before Starting* section) directly to the sample and briefly (< 10 seconds) disperse the sample with a pestle.
3. Immediately pour liquid N_2 into the mortar until the sample is covered. Allow the liquid N_2 to evaporate, then grind the sample with downward force until the sample thaws to a paste (**do not** use a circular grinding motion; doing so will shear the DNA). Repeat this step two additional times.
4. Transfer as much of the sample as possible from the mortar back to the 2.0-ml ***SAND*** tube; wash any remaining sample from the mortar into the empty ***SAND*** tube with 1.4 ml of ***SOLUTION B*** and gently mix at room temperature for at least 5 minutes.
5. Centrifuge the sample at 4,500 × g for 5 minutes and carefully transfer the solution to a new 2-ml centrifuge tube. *Optionally, more nucleic acid can be recovered by washing the soil with an additional 1.4 ml of* ***SOLUTION B*** *and handling separately. This typically increases yields 5-15% depending on the matrix properties. Note that including this additional washing will decrease the number of extractions that can be performed with this kit*. You may discard the solid sample at this point.
6. Add 400 µl of pre-heated (50 °C) ***SOLUTION C*** to the solution from Step 5 and mix by inverting the sample approximately 10 times.
7. Add 200 µl of ***SOLUTION D*** to the solution and mix briefly. Transfer 1 ml of the resulting solution to another 2.0-ml microcentrifuge tube.
8. Add 1 ml of chloroform:isoamyl alcohol 49:1 (user supplied) to each of the two tubes containing solution and shake vigorously for 2 minutes. Centrifuge at 4,500 × g for 10 minutes.
9. Transfer the aqueous (top) phase from the two tubes, each to its own new 2.0-ml microcentrifuge tube. Precipitate by adding 1 volume of isopropanol (approximately 900 µl; user supplied) to each, mix by inverting 25 times, and centrifuge at 16,000 × g for 20 minutes.
10. Discard the supernatants; place the microcentrifuge tubes containing the nucleic acid precipitate on ice, and dissolve the precipitates in 50 µl ***nuclease-free ddH₂O*** each. Combine the two samples into one tube.
11. If the sample is discolored with soil organic matter (8 or lower on the purification color guide shown below):
    a. centrifuge TruRNA spin column at 1,500 × g for 2 minutes; discard the liquid,
    b. rinse the resin by adding 500 µl ***nuclease-free ddH₂O*** to the spin-filter and centrifuging at 1,500 × g for 2 minutes; discard the liquid,
    c. load the discolored nucleic acid sample solution (from step 10) on top of the resin column making sure all of the solution enters the column and does not run down the side of the resin column, centrifuge at 2,000 × g for 3 minutes to recover the nucleic acid solution.

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