DR reagent (Total RNA Isolation Kit)

For research use only

www.fairbiotech.com

Introduction

DR reagent provides an efficient 3 step method to isolate total RNA from tissue, cultured animal/bacterial cells, blood and serum. (For blood samples, additional RBC lysis buffer is required). This unique reagent system ensures total RNA with high yield and good quality from samples of unlimited size. If a larger sample is required, the reagent volume can be scaled proportionately, making this reagent not only very user friendly but also highly versatile. RNA phenol extraction is not required and the entire procedure can be completed in 60 minutes. The total RNA is ready for use in RT-PCR, Northern Blotting, cDNA Synthesis and Mapping.

Kit Contents

Catalog No.	DR0004	DR0050	DR0100
DR Reagent 1	4 ml	50 ml	100 ml
DR Reagent 2	500 µl	8 ml	15 ml

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the FB PR Reagent (Plant Total RNA Isolation Kit) is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

∗mortar and pestle ∗microcentrifuge tubes (RNase free) ∗RNase-free H_20 ∗ß-mercaptoethanol ∗chloroform ∗absolute EtOH for preparing 70% EtOH in H_20 (RNase free) ∗isopropanol

∗For blood samples only, additional RBC lysis buffer is required and may be purchased separately. (RL0100 – 100 ml, RL0500 – 500 ml).

Optional requirements

For complete DNA degradation, Add 2 µl DNAse I (2KU/ml) and 10 µl reaction buffer {300 mM Tris-HCl (pH 7.5), 60 mM $MnCl_2$, 300 µg/ml BSA } to the 50µl final product. Let stand for 10 minutes at room temperature (at 25ºC).

DR reagent (Total RNA Isolation Kit) Protocol

Sample Preparation

Tissue

- Cut off 50 mg of fresh tissue. Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

Cultured Animal/Bacterial Cells

- Transfer cultured animal cells (up to 5 x 10^6) or bacterial culture (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube.
- Centrifuge at 14-16,000 x g for 1 minute and pour off the majority of the supernatant (If more than 1.5 ml of bacterial culture is used, repeat this step).
- Use the remaining supernatant to re-suspend the pellet.

Fresh Blood/Frozen Blood

- Collect blood in EDTA-Na_2 treated collection tubes (or other anticoagulant mixtures).
- Transfer up to 300 µl of blood to a 1.5 ml microcentrifuge tube. If the blood sample is more than 300 µl (up to 1 ml), add the sample to a sterile 15 ml centrifuge tube.
- **Frozen Blood samples** proceed directly to Step 1 Lysis. **If using Fresh Blood samples**, add 3X the sample volume of **RBC Lysis Buffer** and mix by inversion. Do not vortex.
- Incubate the tube for 10 minutes at room temperature.
- Centrifuge for 5 minutes at 3,000 x g and **remove the supernatant completely**.
- Add 100 µl of **RBC Lysis Buffer** to resuspend the cell pellet.

Serum

- Transfer 100 µl of serum to a 1.5 ml microcentrifuge tube.

Step 1 Lysis

Tissue

- Add **500 µl of DR reagent 1** and 8 µl of ß-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved.
- Transfer the dissolved sample to a 1.5 ml microcentrifuge tube. Incubate at 60ºC for 10 minutes.
- Incubate at 15-30ºC for 5 minutes.
- Centrifuge at 14-16,000 x g at 2-8ºC for 10 minutes and transfer the clear supernatant to a new 1.5 ml microcentrifuge tube.

Cultured Animal and Bacterial Cells/Fresh Blood

- Add **500 µl of DR reagent 1** and 8 µl of ß-mercaptoethanol to the sample and mix completely.
- Incubate at 60ºC for 10 minutes then incubate at 15-30ºC for 5 minutes.

Frozen Blood

- Add **500 µl of DR reagent 1** and 8 µl of ß-mercaptoethanol to the sample and mix completely.
- Incubate at 90ºC for 30 minutes then incubate at 15-30ºC for 5 minutes.
- Centrifuge at 14-16,000 x g at 2-8ºC for 10 minutes and transfer the clear supernatant to a new 1.5 ml microcentrifuge tube.

Serum

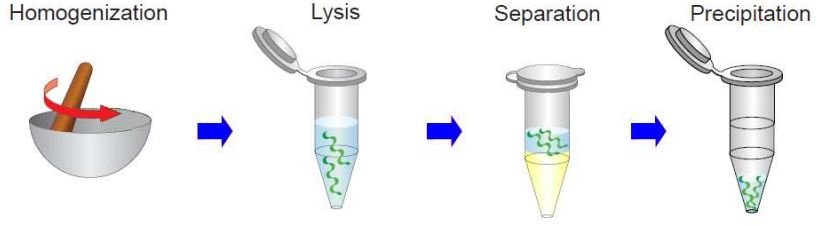
- Add **500 µl of DR reagent 1** and 8 µl of ß-mercaptoethanol and mix completely.
- Incubate at 60ºC for 10 minutes then incubate at 15-30ºC for 5 minutes.

Step 2 Phase Separation

- Add a 1/10 volume of **DR reagent 2** and 500 µl of chloroform to the supernatant from Step 1.
- Shake vigorously and then centrifuge at 2-8ºC at 14-16,000 x g for 10 minutes.
- Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.
- **Repeat the Phase Separation Step until the interphase becomes clear then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube. (**The number of repetitions is dependent on sample type; e.g. dense tissue samples may require a higher number of repeats.)

Step 3 RNA Precipitation

- Add 500 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step 2.
- Mix the sample by inverting gently and Incubate on ice for 10 minutes.
- Centrifuge at 2-8ºC at 14-16,000 x g for 15 minutes. Discard the supernatant and wash the pellet with 1 ml of 70% EtOH.
- Centrifuge at 2-8ºC at 14-16,000 x g for 5 minutes. **Completely discard the supernatant** and re-suspend the pellets in 50-100 µl of RNase-free H_2O. Incubate for 10 minutes at 60ºC to dissolve the pellet.



Troubleshooting

Problem	Cause	Solution
Difficult to dissolve RNA	Incomplete removal of EtOH	Remove EtOH in the hood briefly.
Genomic DNA containment	Incomplete removal of gDNA	DNase treatment.
Degraded RNA/ low integrity	RNases contamination	Work RNases free: Clean everything, use barrier tips, wear gloves and a lab coat. Use RNase-free enzymes and RNase inhibitor.
	Improper sample handling from harvest to lysis	If not processed immediately, freeze the tissue immediately after harvesting, and store it at -80℃ or in liquid nitrogen. Frozen samples must remain frozen until the Lysis Buffer is added. Perform the lysis quickly after adding the Lysis Buffer.
	Tissue highly rich in RNases	Add RNase inhibitors/inactivators to protect the RNA from degradation, or use a larger volume of the DR buffer 1.
RNA containment	Incomplete removal of RNase	RNase A treatment
Low yields of RNA	Incomplete lysis and homogenization	Complete homogenization.
		Cut samples into smaller pieces and ensure the pieces are completely immersed in DR Buffer 1 to achieve the optimal lysis.
	Incorrect precipitation conditions	Add RNase-free H_2O (50~100μL) and incubate for 10 min at 60℃.
	Poor starting material quality	Be sure to use the fresh samples and process immediately after collection or freezing the sample at –80℃ or in the liquid nitrogen immediately after harvesting.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Remove EtOH in the hood briefly.