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RNAZOL® BD PROTOCOL FOR THE ISOLATION OF RNA FROM PLASMA OR SERUM.

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CELL-FREE PLASMA FROM A WHOLE BLOOD SAMPLE

The recovery of RNA from plasma requires caution. After an initial low speed centrifugation step to pellet red and white blood cells, plasma may contain a large number of platelets. Platelets contain a significant amount of RNA so a second centrifugation or filtration step is recommended to obtain "platelet-free plasma".

1. Collect freshly drawn blood in EDTA or citrate (may improve platelet stability) containing blood collection tubes. Process the sample as soon as possible after blood collection (1).
2. Centrifuge the whole blood at 3000 g for 10 minutes.
3. Collect the plasma and filter it through a 0.8 µm Millipore Millex filter (2) to obtain plasma free of red and white blood cells, platelets, and larger apoptotic cell debris.
4. In order to recover vesicles within a particular size range, perform an optional second centrifugation at 10,000 - 20,000 g (2, 3). The centrifugation force will influence RNA and DNA recovery and should be carefully evaluated based on the rotor and centrifuge availability.
5. Add one volume of the "platelet-free plasma" to two volumes of RNAzol BD. Shake the lysate aggressively for at least 30 seconds and store it at -80 C.

SERUM FROM A WHOLE BLOOD SAMPLE

1. Collect freshly drawn whole blood into Becton Dickinsen Serum Vacutainer Tubes and invert to mix.
2. Allow 15 - 30 minutes for clot formation.
3. Recover serum by centrifugation at 1,000 - 2,000 g for 10 - 15 minutes.
4. Decant the serum into a clean tube.
5. The sample may still contain a small numbers of clumped RBC's. Completely remove this potential contamination by filtration or a second centrifugation step. RNA associated with this cellular debris may influence RNA recovery variability.
6. Add one volume of serum to two volumes of RNAzol BD. Shake the lysate aggressively for at least 30 seconds and store it at -80 C.

RNAZOL® BD ISOLATION PROCEDURE

The RNAzol® BD protocol allows for the isolation of total RNA, or the isolation large and small RNA in separate fractions. For instance, mRNA or lncRNA transcripts > 200 bp are isolated in the large RNA fraction while RNA < 200 bp are enriched in the small RNA fraction. For most applications, isolation of total RNA provides the greatest flexibility for use of the isolated RNA.

After obtaining the desired blood fraction as described above, proceed with RNA isolation according to the RNAzol® BD Protocol. Add 10 µl instead of 27 µl of acetic acid per 1 ml of blood sample volume (Step 1). This adjustment addresses the reduced protein content of plasma or serum as compared to whole blood.

Due to the small RNA yield expected from these cell-free preparations, add 2 µl of Polyacryl Carrier (MRC: PC-152) to the aqueous supernatant prior to RNA precipitation with isopropanol. Extend precipitation time in Step 3A or 3B to 20 - 30 minutes at room temperature. Solubilize the RNA in 50 µl of RNase-free water. (See MRC Technical Bulletin 10.)

DNA DIGESTION

Plasma and serum samples contain small, degraded DNA fragments that are difficult to remove during RNA isolation. DNAse digestion is recommended. Follow the standard digestion protocol supplied by the vendor. The amount of recovered RNA should range from 20 - 200 ng/ml of plasma. This amount of RNA is below the detection limit for conventional spectroscopy (i.e. NanoDrop quantification). The RiboGreen fluorescence assay can be used to quantify RNA in the 0 - 50 ng range, but protein contamination or the carrier used to improve RNA recovery can quench the resulting fluorescent signal. In order to obtain accurate quantification with the RiboGreen assay, include appropriate carrier controls in the experimental design.

PRIMER SELECTION

The recovered RNA is fragmented and may not be recognized by routinely used primers. Therefore, we recommend evaluating primers designed to identify different regions of a gene of interest. Primers directed at the middle and 5' end of the gene were most affective at detecting these fragmented transcripts (MRC, unpublished data).

To detect high copy number genes such as GAPDH, B-actin or B2M, the cDNA prepared from these extracts should be diluted 1:20 with water. The cDNA aliquot should be 20 % of the reaction volume to obtain Ct values ranging from 25 - 35. Low copy transcripts may require 30 - 50 cycles of PCR with either undiluted cDNA or 1:2 dilutions for gene detection. After these long periods of amplification, we recommend monitoring the melt points of the resulting products to confirm the fidelity of the resulting product.

REFERENCES

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2. Enderle, D, Spiel A, Coticchia CM, et al. Characterization of RNA from exosomes and other extracellular vesicles isolated by a novel spin column - based method. PloS ONE. 2015 10(8):e136133.
3. Szatanek R, Baran J, Siedlar, M and Baj-Krzyworzeka M. Isolation of extracellular vesicles: determining the correct approach (Review). Intern J of Molecular Med. 2015 36:11-17.