Very reliable methods have been developed to extract RNA from different tissues and cell lines (1, 2, 3). RNA preparation, however, is still cumbersome and time consuming, especially when extracting a large number of cells (>10^7) and cell samples. The isolation of RNA from leucocytic cells is particularly difficult for three reasons: a) high levels of endogenous RNase; b) low amount of RNA (RNA/DNA ratio of 0.08 in granulocytes compared to 4.0 in cultured fibroblasts) (3); c) contamination of the RNA isolate with DNA and protein.

To overcome these problems, we developed a simple and efficient RNA isolation procedure by modifying the method described by Chomczynski and Sacchi (2). Our modified protocol has the following advantages: a) it is simple to perform and needs no major equipment; b) it reduces drastically the volume of costly and hazardous solvents; c) both, small scale (5 x 10^6 cells: RNA yield 30-50 µg) and large scale (1 x 10^8 cells) as indicated in (2). This procedure significantly reduced the volume of solution D (up to 20-fold) and hazardous solvents; c) both, small scale (5 x 10^6 cells) as indicated in (2). This procedure dramatically reduced the volume of solution D (up to 20-fold) and hazardous solvents; c) contamination of the RNA isolate with DNA and protein.

The following detailed step-by-step protocol was tested using various undifferentiated and differentiated leucocytic cell lines (U937, HL60, HEL, MM6), but should be applicable to any cell type growing in suspension. Pellet leucocytic cells from a 50 ml culture flask (1 x 10^9 cells/ml) in a 50 ml Falcon tube. Pour off medium, turn Falcon tube upside down and put it on a kimwipe to drain the pellet. While vortexing the pellet, splash 600 µl solution D (4M guanidinium thiocyanate; 25 mM sodium citrate, pH 7; 0.5% sarcosyl; 0.1% 2-mercaptoethanol; 0.1% antifoam A) to the cells. Vortex until reaching a clear very viscous lysate. Pour the suspension directly into a 1.5 ml Eppendorf cup. Aspirate the lysate into a 2 ml syringe fitted with a 23G-needle and expel it into the tube applying high pressure. Repeat this step 10 times. (Antifoam A prevents foaming while shearing DNA). Add 600 µl 8 M LiCl, mix, add 500 µl precooled (-20°C) isopropanol and mix well (do not vortex). Precipitate RNA for 30 min. at -20°C. Spin down RNA at 11000 x g, 10 min., 4°C and remove supernatant completely. Rinse pellet with 1 ml 70% ethanol and spin again at 13000 x g, 5 min., 4°C. Aspirate supernatant, dry the pellet only slightly and resuspend RNA in DEPC-treated water (100 µl). Measure O.D. and store RNA suspension at -20°C.

A wide range of cell numbers (5 x 10^6 - 10^8 cells) was lysed in always the same volume of solution D (600 µl), instead of adjusting the volume of solution D to the actual cell count (100 µl solution D per 10^6 cells) as indicated in (2). This procedure dramatically reduced the volume of solution D (up to 20-fold) and hazardous solvents; c) contamination of the RNA isolate with DNA and protein.

Figure 1. RNA-Blot. RNA from U937 cells was isolated. Increasing amounts of total RNA (5 µg, 10 µg, 15 µg, 20 µg, 25 µg) were dried in a speed vac. RNA-pellets were dissolved by adding 20 µl of RNA-sample buffer [1 ml freshly prepared RNA-sample buffer contained: 212 µl DEPC-treated H2O; 100 µl 10x RNA-running buffer (0.2 M MOPS, 50 mM sodium-acetate pH 5.3; 5 mM EDTA pH8.0); 175 µl formaldehyde; 500 µl formamide; 3 µl ethidium-bromide (10 mg/ml); 10 µl 10% SDS]. The tubes were shaken on an Eppendorf thermomixer at 65°C for 15 minutes and chilled in ice water. 5 µl RNA-loading buffer (15% Ficoll; 0.5% bromophenolblue) was added and the samples were loaded on a gel (1.2% agarose; 1 x RNA-running buffer; 0.66 M formaldehyde) with narrow slots (1 x 10 mm). RNA in the samples was allowed to settle for 15 minutes before the run (20 V; 16 hours) was started. After separation (gel electrophoresis apparatus GNA200, Pharmacia) RNA was completely transferred onto Nytan nylon membrane (Schleicher and Schuell) by vacuum blotting (Pharmacia) for two hours. The RNA was fixed on the membrane by UV autocrosslinking (Stratagene). The blot was shortly rinsed in distilled water and washed with 2 x SSC, 0.1% SDS at room temperature for about 15 minutes.
for high cell numbers, but resulted in a very viscous lysate. The RNA extractions following the original protocol (2) were then difficult to perform and resulted in impure RNA (Figure 3). Especially phenol/chloroform extractions led to a huge DNA and protein cushion at the interphase that occupied also the aqueous upper phase, making it impossible to recover RNA without contaminants. We simply overcame this problem by initially shearing the cellular DNA with a 23G needle to reduce the viscosity of the lysate (additionally shearing with a 27G needle improves the result). The less viscous lysate was then precipitated with LiCl, resulting in a still impure initial RNA pellet. However, the majority of the contaminants were discarded by aspiration of the supernatant. This step facilitated the subsequent RNA isolation (see above) very efficiently.

Different amounts of total RNA (Figure 1) or total RNA isolated from different sources (Figure 2A) were separated by agarose gel electrophoresis. As can be seen in Figure 1, small as well as large amounts of RNA give excellent sharp 18S and 28S rRNA bands. No DNA contamination is visible. Our method produced excellent RNA isolates from various leucocytic cell lines (Figure 2A). The RNA extracts were further used for measuring mRNA of N-ras, a gene with low expression (Figure 2B) and of β-actin, a gene with high expression Figure 2C).

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