

Overview of Affinity Purification

Affinity purification is a versatile and highly specific technique for the purification of all classes of biomolecules utilizing differences in biological activities of chemical structures. The high selectivity of this technique results in good purification and high recovery. Often a concentrating effect is reached which enables large volumes to be conveniently processed.

This section describes products which are based on hybridization properties of certain nucleic acids.

The mRNA kits rely on base pairing between poly (A⁺) residues at the 3' end of mRNAs and the oligo (dT) residues of a biotin-labeled oligo (dT) probe. The biotinylated dT-A hybrid is bound to streptavidin-coated surfaces of either tubes or magnetic particles. Some of the described kits prepare mRNA directly whereas the other one starts with the purification of total nucleic acid and subsequent isolation of the mRNA.

All of these methods:

- Are much faster than traditional nucleic acid isolation methods
- Minimize nucleic acid handling
- Do not require time-consuming centrifugation or electrophoresis steps
- Avoid the use of toxic organic solvents
- Increase the reliability and reproducibility of RNA isolation from human blood and bone marrow research samples
- Are sensitive enough to isolate mRNA from very rare cells circulating in blood (*e.g.*, disseminated tumor cells)

For a quick overview of each of these products, continue reading this article. Or, for detailed information on the product most relevant to your research, turn to the page that describes the product in detail:

If you are interested in	For preparing	See page
mRNA Capture Kit	poly(A) ⁺ RT-PCR templates from small amounts of total RNA (up to 40 µg), cultured cells (up to 5 x 10 ⁵), or tissue (up to 20 mg), and the simultaneous immobilization in PCR tubes	170
mRNA Isolation Kit	poly(A) ⁺ RNA from larger amounts of total RNA (up to 2.5 mg), cultured cells (up to 10 ⁸), or tissue (up to 1 g)	175
RNA/DNA Stabilization Reagent for Blood/Bone Marrow	Stabilized total nucleic acids from 1.5 – 5.0 ml samples of human whole blood or bone marrow aspirates	183
mRNA Isolation Kit for Blood/Bone Marrow	Highly purified mRNA directly from blood or bone marrow lysates stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow	184

mRNA Capture Kit and mRNA Isolation Kit

The mRNA Capture Kit and the mRNA Isolation Kit are designed to prepare mRNA directly from a variety of starting materials without isolating total RNA. Both can easily process multiple samples simultaneously. Unlike traditional methods, though, the kit procedures take only minutes.

Both kits depend upon the affinity of the poly (A)⁺ tail of mRNA for a biotin-labeled oligo(dT) probe. The probe can “pull” the mRNA selectively from a lysate without interacting with other RNA or DNA. Once formed, the biotinylated dT-A hybrids can be immobilized on solid surfaces that have been coated with streptavidin, then washed free of unbound contaminants.

In the mRNA Capture Kit, the biotinylated dT-A hybrids are bound to streptavidin-coated PCR tubes, where they can be used directly for RT-PCR. In fact, the entire process, from lysate to final PCR can be done in the same tube. The capture process requires approx. 30 min.



The mRNA Capture Kit contains only reagents for the purification of mRNA; it includes neither reverse transcriptase nor reagents for amplification.

In the mRNA Isolation Kit, the biotinylated dT-A hybrids are bound to streptavidin-coated magnetic particles. The particles are immobilized with a magnetic particle separator. The mRNA is readily released from the particles and is pure enough for all downstream applications, including RT-PCR, Northern blotting, Northern ELISA, and *in vitro* translation. The isolation process requires approx. 30 min.

mRNA Isolation Kit for Blood/Bone Marrow

The kit which is intended for general laboratory use relies upon the efficient binding of nucleic acids to silica surfaces in the presence of chaotropic salts and use the silica surface of magnetic glass particles (Figure 45).

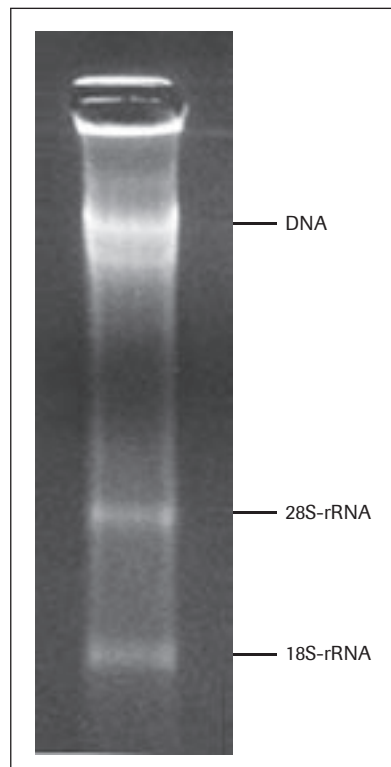


Figure 45: Binding of nucleic acids to magnetic glass particles. Total nucleic acids were isolated with and eluted from magnetic glass particles. Electrophoretic analysis of the eluate (on an agarose gel in the presence of ethidium bromide) shows that it contains DNA as well as 28S- and 18S-rRNA.

Once total nucleic acids are collected on and released from magnetic glass particles, they can be subfractionated. In the kit, poly(A)⁺ RNA is isolated from total nucleic acids with biotin-labeled oligo(dT)₂₀ and streptavidin-coated magnetic particles. The two-step approach (first collect total nucleic acids, then isolate mRNA) produces a highly purified, highly concentrated mRNA preparation.

Stabilization of total nucleic acids followed by mRNA isolation

The approach involves two distinct steps: Whole blood samples or bone marrow aspirates (research samples) are lysed and instantaneously stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. Then, mRNA is isolated from the stabilized lysate with the mRNA Isolation Kit for Blood/Bone Marrow. The advantages of this approach are:

- This method involves no cell separation step; thus, there is no danger of losing rare cells that show an aberrant sedimentation or lysis behavior.
- If a sample is mixed with the RNA/DNA Stabilization Reagent immediately after it is drawn, the mRNA in the sample is instantly protected from degradation, even if the stabilized lysate is stored or transported to another location for processing and analysis.

The characteristics of the method is summarized in the following table:

Characteristic*	mRNA Isolation Kit for Blood/Bone Marrow
Starting Material	Blood or bone marrow stabilized with RNA/DNA Stabilization Reagent for Blood/Bone Marrow (up to 5 ml)
Removal of RBCs	Not required
Lysis of WBCs	Not required
Total NA isolation with MGPs	Required
mRNA isolation with SMPs	Required
Final mRNA elution volume	20 µl

* Abbreviations: MGPs, magnetic glass particles; NA, nucleic acids; RBCs, red blood cells; SMPs, streptavidin-coated magnetic particles; WBCs, white blood cells

Advantages of the kit

- Flexible enough to handle a range of sample sizes
- Sensitive enough to detect rare mRNAs
- Powerful enough to remove inhibitors



We estimate that, to obtain a positive RT-PCR from a research blood sample containing tumor cells (one cell/ml, each with 10 specific mRNA transcripts), you would need to amplify the mRNA from at least 2 ml of whole blood.

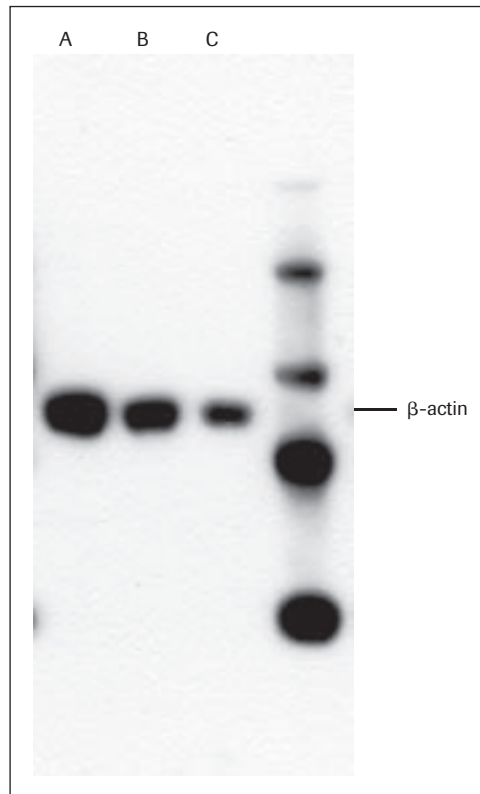


Figure 46: Flexibility of the mRNA Kit. The mRNA Isolation Kit for Blood/Bone Marrow was used to isolate mRNA from different volumes of heparinized human blood research samples. An aliquot (30%) of each mRNA was separated electrophoretically, transferred to a membrane by blotting, then analyzed with a DIG-labeled antisense β -actin RNA probe.

Lane A: mRNA from 5 ml blood

Lane B: mRNA from 3 ml blood

Lane C: mRNA from 1.5 ml blood

Result: Each isolated mRNA contained a strong actin band; the amount of the band varied according to the amount of starting material. There was no evidence of degradation products in the preparation.

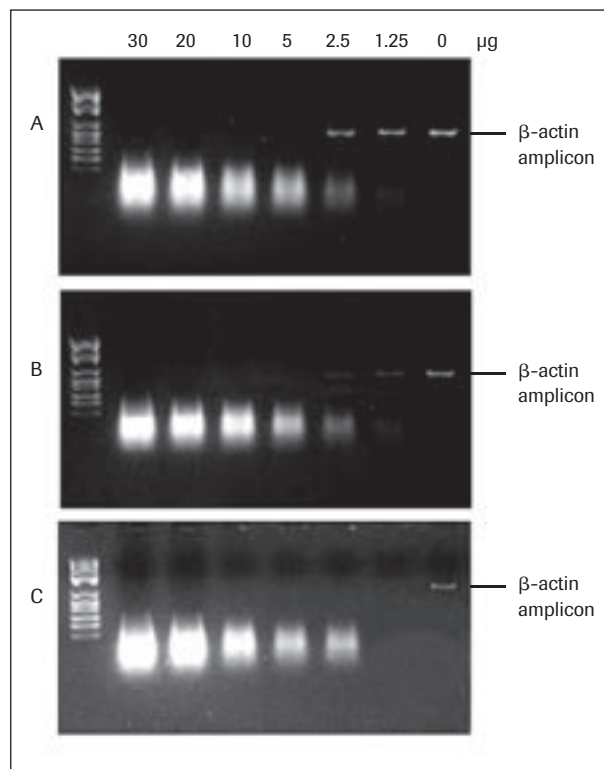


Figure 47: The effect of rRNA on RT-PCR. Varying amounts of β -actin mRNA (1 ng, panel A; 40 pg, panel B and 1.6 pg, panel C) were mixed with increasing amounts of rRNA, as indicated. The mixtures were used as templates for RT-PCR.

Result: The presence of 2.5 μ g rRNA (less than the amount in 1 ml blood) completely inhibited the specific amplification of β -actin mRNA. Yet, in the absence of any rRNA, a β -actin amplicon was produced from only 1.6 pg mRNA (equivalent to the mRNA from 1.6 cells).