



## mRNA Directly from Lysates

### chemagic mRNA Direct Kit

The **chemagic mRNA Direct Kit** is designed for the direct purification of high quality mRNA from crude extracts of animal tissues, cells and plants. The direct isolation is performed without having to previously prepare total RNA while maintaining the high yield and purities of the resultant mRNA.

The standard **chemagic mRNA Direct Kit** contains sufficient reagents for at least 50 isolations of mRNA directly from  $10^7$  cells, 100 mg animal tissue or 100 mg plant tissue.

The method relies on the base pairing between the polyA\* tail of most messenger RNAs and the oligo dT sequence bound to chemagen's proprietary M-PVA Magnetic Bead surface. Comparison of the purity of mRNA isolated from common samples using other available market kits with the chemagic mRNA Direct Kit, showed that chemagen's kit yielded the purest mRNA.

- Sample size: scalable.
- Sample sources: cell lines, plants, tissue.
- Preparation time: 30 - 40 minutes.
- Typical yields: e.g. 3 - 5  $\mu$ g mRNA (from cell culture containing  $10^7$  cells).

### I. mRNA Isolation from Cultured Cells

PolyA\* RNA was isolated manually from cells applying the **chemagic mRNA Direct Kit** and two different competitor kits. The competitor B kit requires a previous total RNA isolation step.

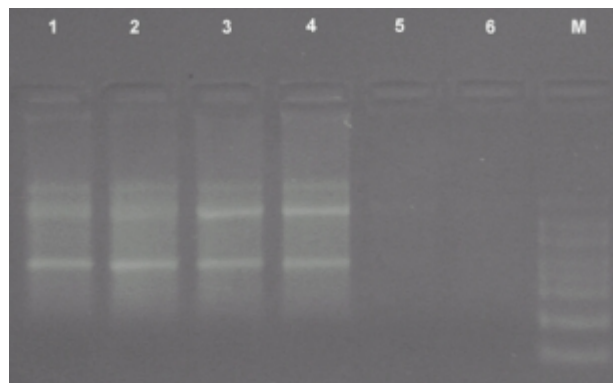


Fig. 1: Direct polyA<sup>+</sup> RNA isolation from mouse hybrid cells ( $5 \times 10^6$ ) using three different kits. Formaldehyde agarose gel analysis using 25  $\mu$ l of the eluted mRNA (= 1/4). **1 - 2 chemagic mRNA Direct Kit.** **3 - 4** mRNA purification system from competitor A. **5 - 6** mRNA purification system from competitor B. M: 100 bp ladder.

*Further Questions?*

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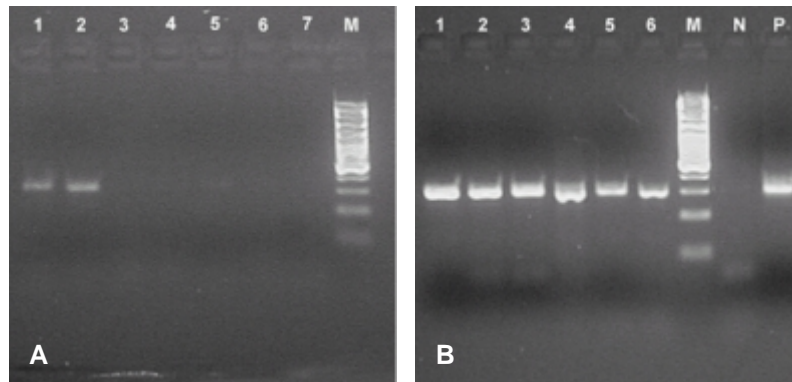


Fig. 2: RT-PCR of polyA<sup>+</sup> RNA isolated with three different kits; **1 - 2 chemagic mRNA Direct Kit**. **3 - 4** mRNA purification system from competitor A. **5 - 6** mRNA purification system from competitor B. Ten (10) µl of the bead/resin suspension (= 1/10) was reverse-transcribed using the bead/resin-bound oligo-dT that binds to the polyA-tail of the mRNA as the primer for first-strand cDNA synthesis. Two (2) µl of the resulting solid-phase cDNA library was used as a template for a full PCR-amplification of glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Panel **A**) or the zinc-binding protein parathymosin- $\alpha$  (ZnBP, Panel **B**). **M** 100 bp ladder. **P** positive control (amplification of genomic DNA) and **N** negative control without RT-enzyme (no DNA contamination is detected).

## II. mRNA Isolation from Tissue

PolyA<sup>+</sup> RNA was isolated manually from tissue samples. Again, regarding competitor kit B, a total RNA purification step was required before isolating PolyA<sup>+</sup> RNA.

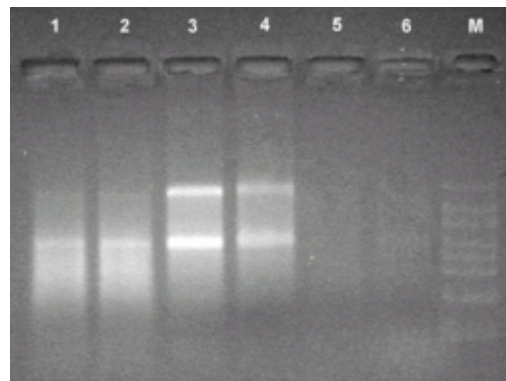


Fig. 3: Direct polyA<sup>+</sup> RNA isolation from mouse liver (50 mg) using three different kits. Formaldehyde agarose gel analysis using 25 µl of the eluted mRNA (=1/4). **1 - 2 chemagic mRNA Direct Kit**. **3 - 4** mRNA purification system from competitor A. **5 - 6** mRNA purification system from competitor B. **M** 100 bp ladder.

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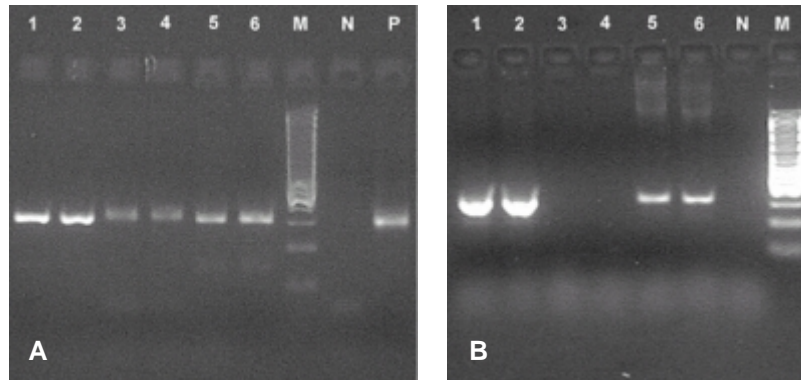


Fig. 4: RT-PCR of polyA<sup>+</sup> RNA isolated with three different kits; **1 - 2 chemagic mRNA Direct Kit**. **3 - 4** mRNA purification system from competitor A. **5 - 6**: mRNA purification system from competitor B. Ten (10)  $\mu$ l of the bead/resin suspension (= 1/10) was reverse-transcribed using the bead/resin-bound oligo-dT that binds to the polyA-tail of the mRNA as the primer for first-strand cDNA synthesis. Two (2)  $\mu$ l of the resulting solid-phase cDNA library was used as a template for a full PCR-amplification of glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Panel **A**) or the zinc-binding protein parathymosin- $\alpha$  (ZnBP, Panel **B**). **M** 100 bp ladder. **P** positive control (amplification of genomic DNA) and N: negative control without RT-enzyme (no DNA contamination is detected).

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