mTRAP™

grab onto higher yields of pure mRNA

mTRAP Kits utilize gripNAs,™ an advanced form of negatively charged Peptide Nucleic Acids (PNAs), to capture and purify mRNA from cells, tissues or total RNA.

mTRAP advantages

- Higher yields of pure mRNA
- Less ribosomal RNA and genomic DNA contamination
- More representative mRNA populations
- Optional DNase treatment for sensitive applications

ACTIVE MOTIF®

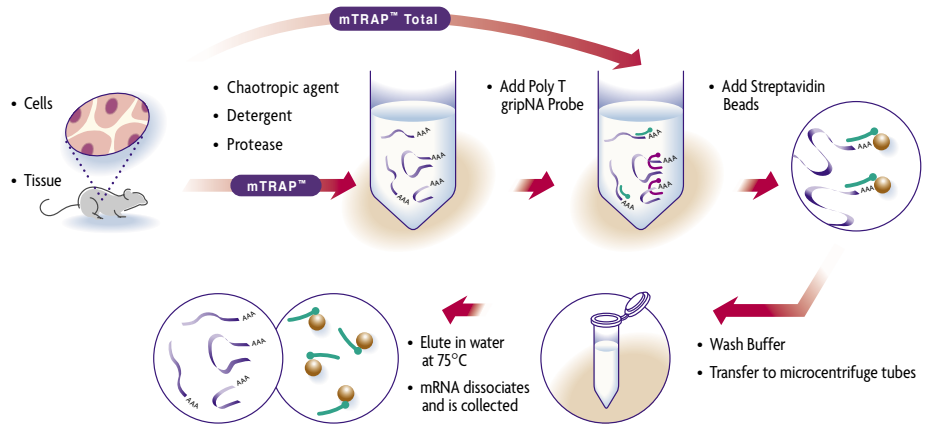
Tools to Analyze Nuclear Function

Products: mTRAP Maxi Kit | mTRAP Midi Kit | mTRAP Total Kit

mTRAP™ – grab onto higher yields of pure mRNA

mTRAP™ Kits utilize gripNAs,™ an advanced form of negatively charged Peptide Nucleic Acids (PNAs),* to capture and purify mRNA from cells, tissues or total RNA samples. gripNAs bind nucleic acids with extremely high affinity and specificity, which results in higher yields of mRNA with reduced ribosomal RNA (rRNA) and genomic DNA contamination. Plus, the high affinity of gripNAs enables isolation of more representative mRNA populations, including mRNA molecules with complex secondary structures and short poly A tails.

Conventional mRNA isolation methods hybridize oligo dT to the 3´ poly A tails of mRNA. However, this relatively weak DNA/RNA interaction must be stabilized through the use of high-ionic strength buffers, which can also preserve the complex secondary structures of the RNA molecules. Consequently, the poly A tails of these mRNA are inaccessible for oligo dT binding, limiting the efficiency of isolation and resulting in lower yields. These conditions can also promote the non-specific binding and subsequent co-purification of ribosomal RNA (rRNA) and genomic DNA, which may interfere with downstream applications such as microarray analysis. Furthermore, the low affinity of oligo dT for poly A RNA means that these methods often fail to isolate mRNA molecules with shorter poly A tails (< 20 bases).



Flowchart of the mTRAP procedure

The mTRAP advantage

High-affinity Poly T gripNA Probe

To overcome the problems associated with mRNA purification, Active Motif has developed an alternative method that uses PNAs. PNAs are DNA analogs in which the nucleosides are attached to an *N*-(2-aminoethyl) glycine backbone instead of to deoxyribose, as in DNA.[1-4] While classical PNAs have a higher affinity for nucleic acids than oligo dT (Table 1), poor solubility and a tendency to self-aggregate have limited their use.

Active Motif has overcome these shortcomings by developing gripNAs, a novel form of negatively charged PNAs.[5-7] gripNAs have similar binding properties to classical PNAs but are superior in terms of solubility and ease of handling. Further development has led to the selection of two distinct analogs, a (T) phosphono PNA (pPNA) and a (T) *trans*-4-hydroxy-L-proline (HypNA). When combined in a 1:1 ratio (Figure 1), these analogs have a number of properties that make them ideal for mRNA isolation. mTRAP Kits utilize a Poly T gripNA Probe that provides many benefits over oligo dT-based methods:

- Higher yields of pure mRNA
- Less rRNA and genomic DNA contamination
- More representative mRNA populations
- Optional DNase treatment for sensitive applications

Table 1: Differential nucleic acid binding affinities (T_m) of gripNAs

	16-mer poly-A DNA	16-mer poly-A RNA
Oligo dT DNA	46.0°C	47.4°C
Classical Poly T PNAs	85.0°C	82.0°C
Poly T gripNA Probe	81.5°C	72.5°C

Higher yields of pure mRNA
mTRAP Poly T gripNA Probe contains T-pPNA and T-hypNA monomers in a 1:1 ratio that confers extremely tight and specific binding of poly A mRNA. This high-affinity binding enables the use of low-salt Lysis, Wash and Elution Buffers that destabilize mRNA secondary structures as well as any weak, non-specific interactions that may have formed between nucleic acids and proteins with the Poly T gripNA Probe. The result is significantly higher yields of pure mRNA with lower levels of rRNA contamination compared to oligo dT-based methods (Figures 2 & 3).

More representative mRNA populations
The higher affinity of the mTRAP Poly T gripNA Probe for poly A RNA together with the low-salt conditions enable the isolation of mRNAs with shorter poly A tails and with complex secondary structures. The result is a more representative population of mRNA molecules in your sample.

Less genomic DNA contamination
A further advantage of the Poly T gripNA Probe is conferred by its differential affinity (ΔT_m: 9°C) for DNA and RNA (Table 1). This property allows the selective purification of mRNA from any bound genomic DNA during a 75°C elution step. High-temperature elution ensures complete recovery of mRNA free from genomic DNA contamination.

Optional DNase treatment for sensitive applications
Unlike oligo dT, mTRAP Poly T gripNA Probe is resistant to enzymatic degradation. This allows you to perform an optional DNase step during isolation that eliminates even trace amounts of genomic DNA for better results in even the most sensitive downstream applications.

* Patent pending

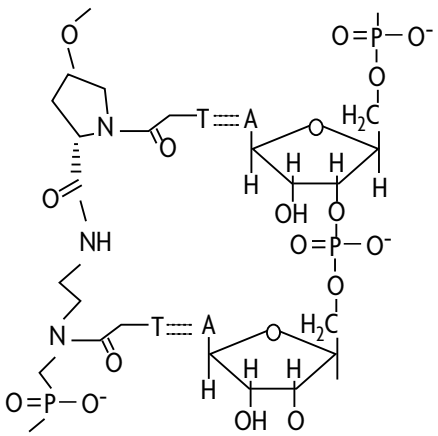


Figure 1: Negatively charged PNA analogs.
The Poly T gripNA Probe, comprised of alternating T-pPNA and T-HypNA analogs, hybridized to the poly A tail of an mRNA molecule.

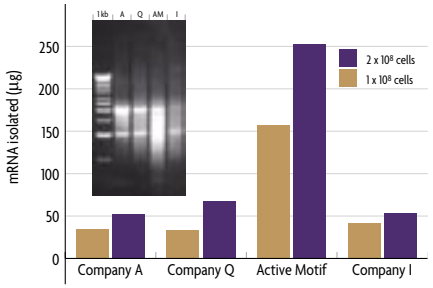


Figure 2: Higher yields of pure mRNA.
mRNA was isolated from samples of 1 and 2 x 10^8 HeLa cells using mTRAP Maxi and three other suppliers' kits. Eluted mRNA was quantified by a spectrophotometer and plotted. Two µg of mRNA from each 1 x 10^8 sample was run on a 0.8% agarose gel. mTRAP™-isolated mRNA shows no genomic DNA and less rRNA.

Lane 1:	EXACT 1 kb DNA Ladder
Lane 2:	mRNA isolated using Company A's kit
Lane 3:	mRNA isolated using Company Q's kit
Lane 4:	mRNA isolated using mTRAP™ Maxi Kit
Lane 5:	mRNA isolated using Company I's kit

Figure 3: Pure mRNA from total RNA in one passage.
mRNA was isolated from 500 µg of total RNA in duplicate, quantified by spectrophotometry and run on a 0.8% agarose gel.

Lane 1:	EXACT 1 kb DNA Ladder
Lanes 2 & 3:	2 µg mRNA isolated in one pass with the mTRAP Total Kit.

Kit Specifications

Sample	mTRAP Maxi Kit	mTRAP Midi Kit	mTRAP Total Kit
Cells	0.5-2.0 x 10^8	0.5-1.0 x 10^7	–
Tissue	0.4-1 g	50-200 mg	–
Total RNA	–	–	500 µg
Yield	100-200 µg*	10-20 µg*	10-15 µg

* Depending on cell type

References:

1. Nielsen P.E. *et al.* (1991) *Science* 254: 1497-1500.
2. Egholm M. *et al.* (1992) *J. Am. Chem. Soc.* 114: 1895-1897.
3. Harvey J.C. *et al.* (1992) *Science* 258: 1481-1485.
4. Buchardt O. *et al.* (1993) *Trends Biotechnol.* 11: 384-386.
5. Efimov V.A. *et al.* (1998) *NAR* 26: 566-575.
6. van der Laan *et al.* (1996) *Tetrahedron Lett.* 37: 7857-7860.
7. Efimov V.A. *et al.* (1999) *NAR* 27: 4416-4426.

Ordering Information

Product	Format	Catalog No.
mTRAP™ Maxi	6 rxns	23006
	5 x 6 rxns	23506
mTRAP™ Midi	24 rxns	23024
	5 x 24 rxns	23524
mTRAP™ Total	12 rxns	23012
	5 x 12 rxns	23512
Poly T gripNA Probe (Maxi Kit)	6 rxns	29007
Poly T gripNA Probe (Midi Kit)	24 rxns	29008
Streptavidin Beads (Maxi Kit)	3 ml	29009
Streptavidin Beads (Midi Kit)	1.6 ml	29010
mTRAP™ Lysis Buffer	100 ml	29011
Protease	20 mg	29012