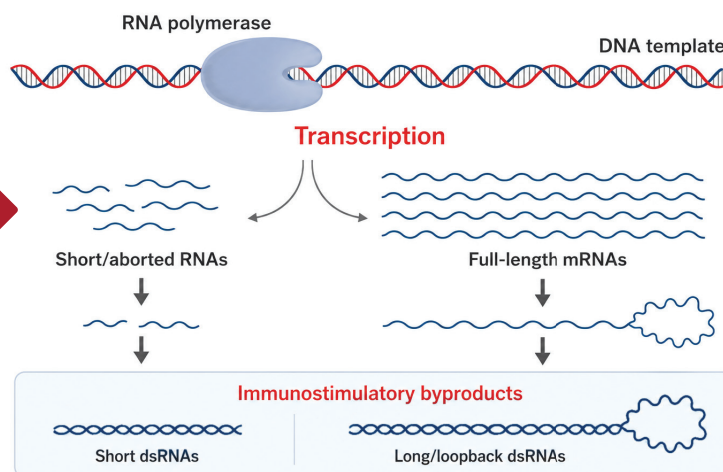


The hidden trigger in mRNA

High-quality *in vitro* transcribed (IVT) mRNA depends on three fundamentals: optimized mRNA design, a robust mRNA synthesis workflow, and comprehensive quality control—including detection and removal of double-stranded RNA (dsRNA) contaminants. dsRNA is a byproduct contamination in IVT reactions causing increased cellular toxicity and a negative immune response.

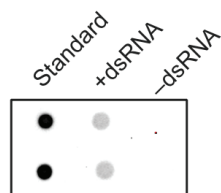
During IVT, RNA polymerases can generate unintended antisense transcripts and self-complementary structures that anneal to form dsRNA contaminants.



How to detect dsRNA

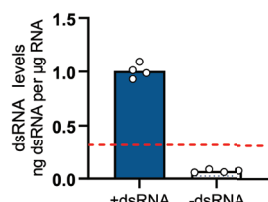
Qualitatively

dsRNA dot blot



Quantitatively

dsRNA ELISA



Antibody-based methods enable qualitative detection of dsRNA by dot blot or quantitative measurement by ELISA.

When delivering mRNA to **sensitive cells or animal models**, dsRNA can activate several signaling pathways:

- MDA5/RIG-1 and TLR3 activation for **antiviral and cytokine signaling**
- PKR activation causing **translation and cell growth inhibition**
- Inflammasome assembly leading to **cell death**

Removing dsRNA from IVT mRNA leads to:

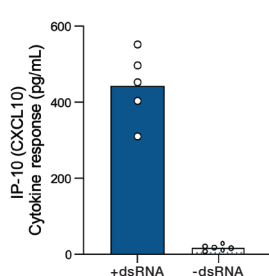
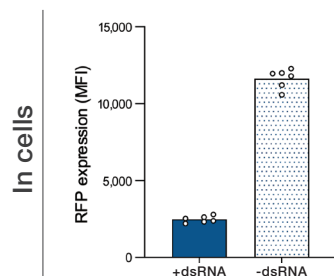
↑ higher expression

↓ reduced inflammation



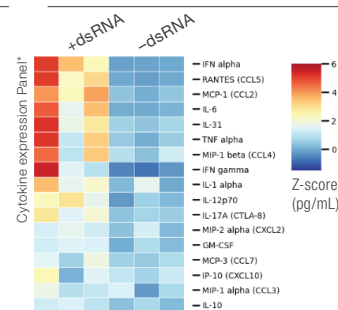
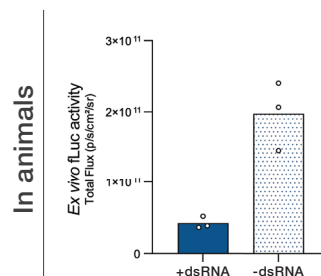
Expression

Inflammation



Expression

Inflammation

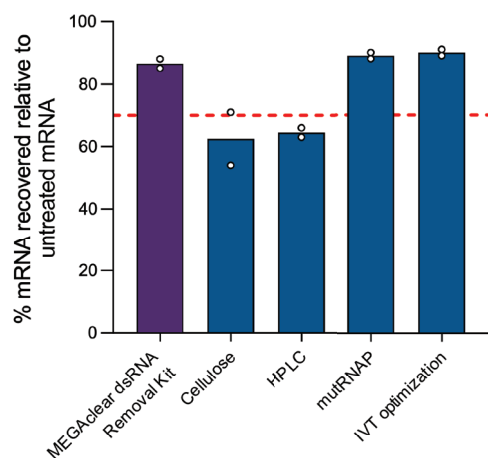


How do you reduce the amount of dsRNA in IVT mRNA?

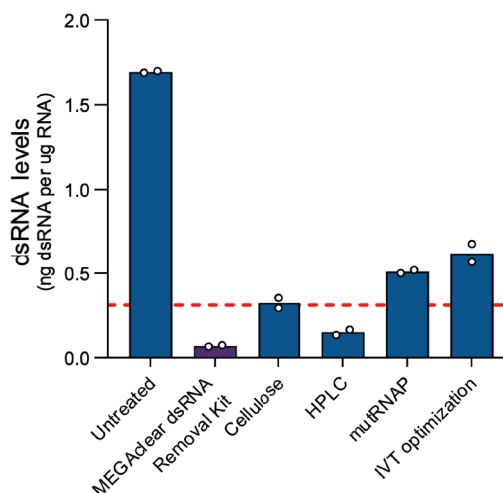
Approaches	Post-IVT purification			IVT reaction	
Methods	Invitrogen™ MEGAclean™ dsRNA Removal Kit	Cellulose	IP-RP HPLC	Mutant RNA polymerase	IVT optimization
dsRNA removal	+++	++	+++	+	+
mRNA yield	+++	+	+	+++	+++
Ease of use	+++	++	+	+++	+++

+++ : excellent ++ : good + : fair

mRNA yield



dsRNA presence



MEGAclean dsRNA Removal Kit

- ✓ HPLC performance. Bench-top simplicity
- ✓ Lower dsRNA impurities, higher mRNA recovery
- ✓ Clean mRNA for more reliable biology
- ✓ Purification that scales with your research.



Ordering information

Product description	Product name	Unit size	Cat. No.
dsRNA removal	Invitrogen™ MEGAclean™ dsRNA Removal Kit	25 reactions	A56871
		1,000 reactions	A56872
In vitro transcription kit	Invitrogen™ mMESSAGE mMACHINE™ T7 mRNA Kit with CleanCap™ Reagent AG	25 reactions	A57620-25
		50 reactions	A57620
		1,000 reactions	A57621
mRNA purification	Invitrogen™ MEGAclean™ Transcription Clean-Up Kit	20 preps	AM1908
		2 mL	65030D
	Invitrogen™ Dynabeads™ RNA Purification Kit	10 mL	65032D

Learn more at thermofisher.com/dsrna