Produce Ψ-mRNA with reduced dsRNA in one Complete system

INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System

Product Intro

The INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System provides all reagents for making Ψ-mRNA with greatly reduced double-stranded RNA (dsRNA) content and nearly 100% 5′ capping. The Complete kit combines high-yield transcription incorporating ΨTP, post-transcriptional 5′ capping and 3′ poly(A) tailing, and enzymatic dsRNA removal to <0.005% (LLOQ). INCOGNITO™ Complete Ψ-mRNA is suitable for use in transfection and microinjection experiments as well as *in vitro* translation systems.

Benefits

- **Lower immunogenicity:** Synthesize transcripts with ΨTP and low dsRNA for reduced immune response.
- **Complete solution:** Combine transcription, 5′ capping, 3′ tailing, and dsRNA removal in one system.
- **High efficiency capping:** Achieve ~100% 5′ Cap 1 capping.
- **Customizable poly(A) tailing:** Create variable 3′ poly(A) tail lengths, from 50 to >300 bases long.
- **Effective dsRNA removal:** Eliminates dsRNA to <0.005% (LLOQ) of sample.

Product Description

The INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System provides all enzymes and reagents for making pseudouridine (Ψ)-containing, 5′-capped, 3′-polyadenylated mRNA with greatly reduced double-stranded RNA (dsRNA) content. It includes reagents for five workflow modules, (1) RNA transcription, (2) 5′ capping, (3) 3′ poly(A) tailing, (4) enzymatic removal of dsRNA and (5) RNA/mRNA purification:

1. **Transcription:** *In vitro* transcription of linear double-stranded DNA templates using the T7 mScript™ Enzyme Solution with supplied ΨTP, ATP, CTP and GTP.
2. **5′ Capping:** Enzymatic capping of the RNA using ScriptCap™ Cap 1 Capping System (for making mRNA with a Cap 0 cap structure, or optionally making mRNA with a Cap 1 cap structure) (Figure 1, see next page).
3. **3′ Poly(A) Tailing:** A-Plus™ Poly(A) Polymerase for generating 3′ poly(A) tails of 150-200 bases or, using a modified protocol, tail lengths of >300 bases are possible.
4. **dsRNA Removal:** Enzymatic removal of dsRNA content using Min-Immune™ Gold dsRNA Removal system.
5. **Purification:** 5M NH_4OAc is included as a convenient RNA and mRNA purification method.

Figure 1A: Cap 0-mRNA

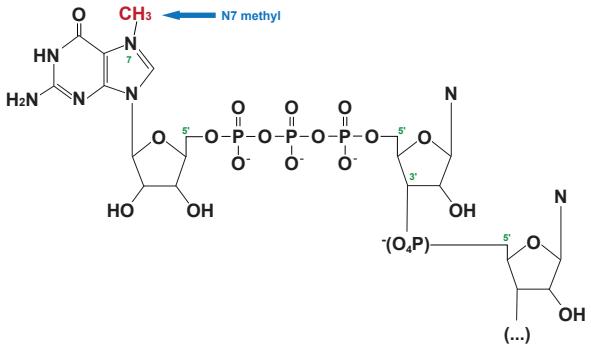

Figure 1B: Cap 1-mRNA

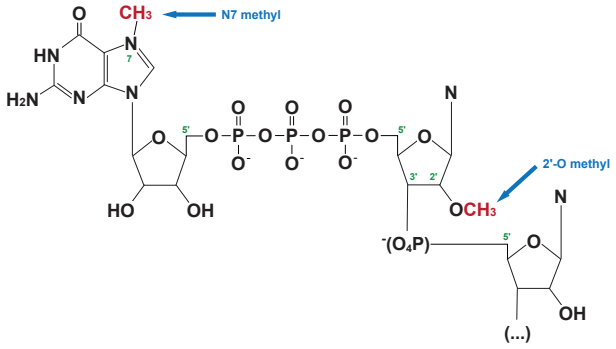

Figure 1: Cap 0-RNA and Cap 1-RNA. Cap 0 is added using ScriptCap™ Capping Enzyme and converted to Cap 1 using 2′-O-Methyltransferase.

Post-transfection, capped and tailed mRNA has increased stability and translation efficiency in most eukaryotic cell lines. The mScript™ System improves upon existing capping methods by ensuring virtually 100% transcript capping, all caps in the proper orientation and the ability to produce large amounts of capped RNA at a reasonable cost. Additionally, the A-Plus™ Poly(A) Polymerase protocol provides a wide range of poly(A) tail lengths from 150–300+ bases. INCOGNITO™ Complete Ψ-mRNAs increase translation and decrease innate immune responses from mammalian cells that express various RNA sensors.

Product Performance

The INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System is functionally tested under standard reaction conditions using the T7 Control Template DNA. The *in vitro* transcription module must produce at least 50 µg of RNA from 1 µg of the T7 Control Template DNA in 20 minutes at 37°C. A-Plus™ Poly(A) Polymerase is functionally tested in 1X A-Plus™ Poly(A) Tailing Buffer with 1 mM ATP and a 1.4 kb transcript. The capping and dsRNA removal module enzymes are tested independently using non-T7 control transcript RNA to assay for completeness of reaction.

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Ordering information

Catalog Number	Description
IMCY250125	INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System (25 reactions)

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