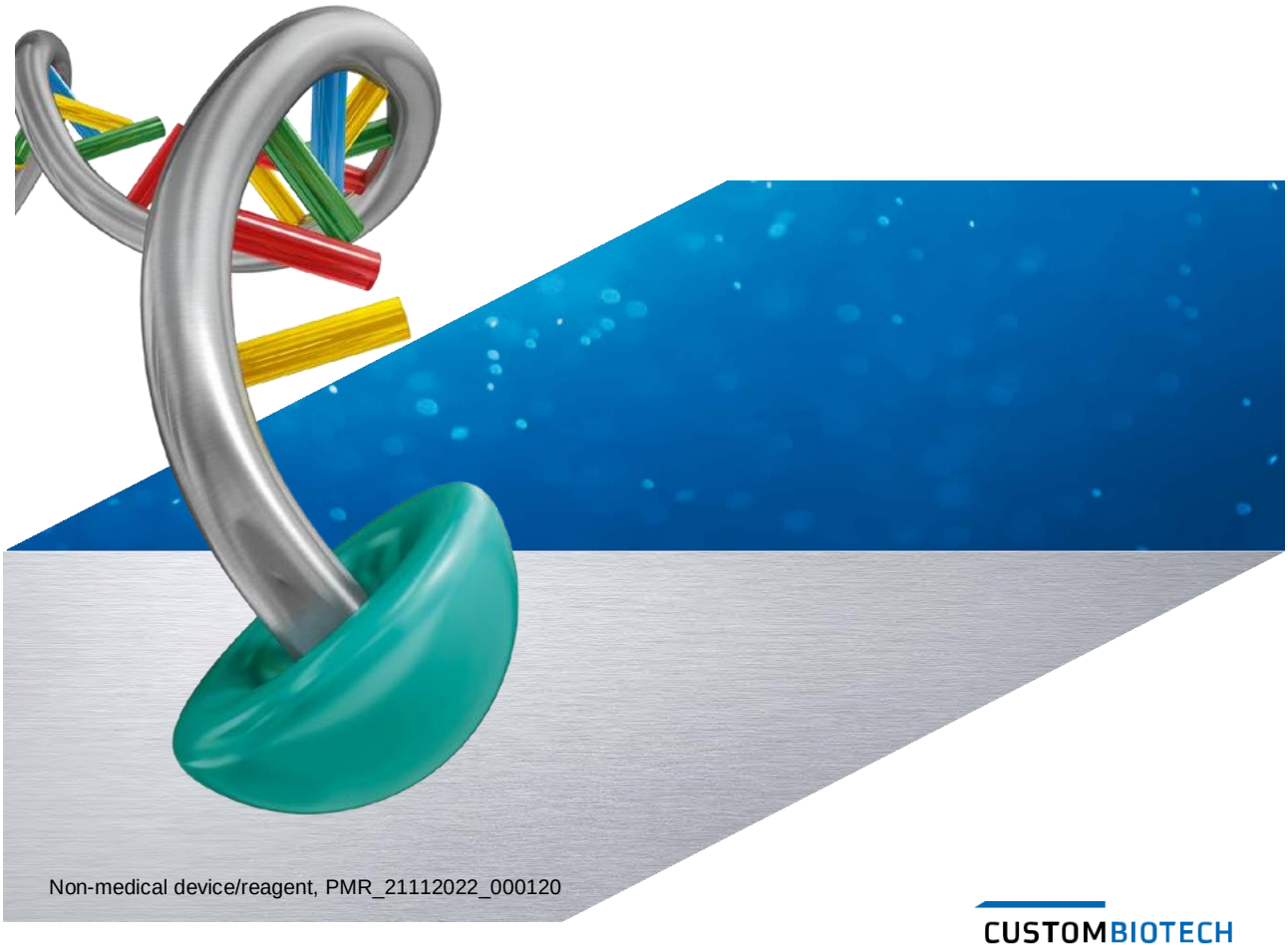
Roche CustomBiotech
– IVT reaction starting condition

*해당 자료는 일괄 확립된 프로토콜이 아닌 IVT 시작 시 참고할 수 있는 가이드라인이므로, 각 실험자의 실험조건에 맞도록 optimization 되어야 합니다.



Non-medical device/reagent, PMR_21112022_000120

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IVT Reaction protocol

IVT reaction protocol (starting conditions)

The following conditions may be a good start:

- 10x Transcription Buffer (basic buffer): 400 mM Tris-HCl (pH 8.0), 20 mM spermidine, 100 mM DTT
- Reaction set up: 20 uL reaction volume; 500 ng template; 1 hr incubation at 37 C
- Reagents used (all Roche CB reagents):
- 1 µL T7 RNA Polymerase
- 10 mM each NTP
- 1 µL PPase
- 20 U RNase Inhibitor
- 40 mM MgCl2

Nucleotide and Mg2+ concentration has a high influence on the reaction. As a general guideline it is better to keep the ratio of NTP:Mg2+ between 0.7-1, during the titration. Alternative 10x Transcription Buffer (not Tris based) : 1 M HEPES-KOH (pH 7.5), 20 mM Spermidine, 400 mM DTT.

When optimizing the conditions, the customers may vary the ratio of the NTPs from 1:1:1:1 to something slightly different. Often, GTP is used in somewhat higher concentration.
UTP can be replaced entirely by N1-Me-Pseudo-UTP (Me-psUTP), or a certain percentage of UTP along with Me-psUTP. This really depends on careful optimization. The amount needed of each NTP depends on the target sequence and further optimizing the process to maximize mRNA yield.