Surrogate RNA 1 equimolar 25℃	68.1	5.0	12.3	21.6
Surrogate RNA 1 alpha 25℃	97.6	0.2	1.9	0.4

Example 10. dsRNA ELISA indicates presence of dsRNA

hEPO RNA construct was generated using equimolar or alpha processes and purified either by oligo dT resin only (-RP) or with reverse phase purification (+RP). ELISA using dsRNA-specific antibodies is used to determine relative differences in the purities of RNAs generated using different processes. RNAs generated using equimolar process contain significantly more dsRNA than RNAs generated with alpha process (FIG. 39). Reverse phase purification improves the purities of -RP RNAs.

Example 11. Radioactive sequencing gel analysis determines sample purity

A short model RNA construct (surrogate RNA 1) was generated using equimolar or alpha processes. Each IVT reaction contained either ^{32}P-GTP, which labels abortive transcripts, or ^{32}P-CTP, which labels reverse complement transcripts. RNA samples were analyzed by sequencing gel. Based on the ^{32}P-GTP data, there is no difference in abortive transcript abundance between the two processes. Based on the ^{32}P-CTP data, equimolar process generates more reverse complements than alpha process.

Example 12. In vivo analysis of dsRNA doped into mRNA

Reverse phase purified hEPO mRNA was doped with 5%, 0.5% or 0.05% w/w 60bp dsRNA, which corresponds to the first 60 nucleotides of the intact hEPO construct. The mRNA samples were formulated in MC3 then dosed by IV into C57BL/6 and Balb-c mice at 0.5 mpk. After 24 hours, the spleens were harvested and homogenized to generate single cell suspensions. The splenic cells were stained for B cell markers then analyzed by flow cytometry. The activated B cell populations were analyzed based on their expression of CD86 and CD69 markers. The 60bp dsRNA has the following sequence:

60mer 5' UTR Epo F GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGGGAGUGCACG (SEQ ID NO. 31)

60mer 5' UTR Epo R CGUGCACUCCCAUGGUGGCUCUUAUAUUUCUUCUUACUCUUCUUUUCUCUCUUAUUUCCC (SEQ ID NO. 32)

Groups that received hEPO mRNA generated by alpha process had lower B cell activation than groups that received hEPO with doped dsRNA. Serum was also collected at 6 h and analyzed by a cytokine luminex panel. The expression trends for IP-10, IFN-gamma, and IFN-alpha markers from the luminex panel were consistent with the trends from the B cell activation analysis, indicating the dsRNA in the doped samples triggered the type I interferon pathway, which led to B cell activation.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All references, including patent documents, disclosed herein are incorporated by reference in their entirety.

What is claimed is:

CLAIMS

1. A method of producing messenger RNA that comprises an open reading frame, the method comprising:

(a) forming a reaction mixture comprising a DNA template and nucleoside triphosphates (NTPs) including adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP), guanosine triphosphate (GTP), or an analog of each respective nucleoside, and a buffer; and

(b) incubating the reaction mixture to produce a composition comprising messenger RNA,

wherein the ratio of concentration of GTP to concentration of ATP is at least 2:1, the ratio of concentration of GTP to concentration of CTP is at least 2:1, and the ratio of concentration of GTP to concentration of UTP is at least 2:1.

2. The method of claim 1, wherein the reaction mixture comprises GTP and GDP and wherein the ratio of concentration of GTP plus GDP to the concentration of any one of ATP, CTP or UTP is at least 2:1.

3. The method of claim 2, wherein the ratio of concentration of GTP plus GDP to concentration of ATP, CTP and UTP is at least 3:1, at least 6:1 and at least 6:1, respectively.

4. The method of claim 1, wherein the reaction mixture further comprises guanosine diphosphate (GDP).

5. The method of claim 1, wherein the composition is substantially free of reverse complement transcription product.

6. The method of claim 1, wherein greater than 90% of the mass of the messenger RNA comprises single stranded full length transcripts.

7. The method of claim 1, wherein the composition is substantially free of RNase III insensitive fragments.

8. The method of claim 1, wherein the composition comprises a population of single stranded partial RNA transcripts in a sense orientation and wherein greater than 80% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or shorter.

9. The method of claim 8, wherein greater than 90% of the population of single stranded partial RNA transcripts in a sense orientation has a nucleotide length of 100 nucleotides or shorter.

10. The method of claim 1, wherein less than 0.25% of the mass of the RNA in the composition is cytokine-inducing RNA contaminant.

11. The method of claim 10, wherein the composition is substantially free of cytokine-inducing RNA contaminant.

12. The method of claim 1, wherein less than 0.5% of the mass of the RNA in the composition is reverse complement transcription product.

13. The method of claim 12, wherein the reverse complement transcription product is a dsRNA comprising a strand comprising a sequence that is a reverse complement of at least a portion of the messenger RNA or a dsRNA comprising a strand comprising a polyU containing sequence.

14. The method of claim 13, wherein the strand comprising the sequence that is the reverse complement of the messenger RNA or the strand comprising the polyU sequence initiates with a 5’ triphosphate (5’-PPP).

15. The method of claim 12, wherein the reverse complement transcription product comprises a reverse complement of the 5’-end of the messenger RNA and/or a reverse complement of the 3’-end of the messenger RNA encoding a polypeptide of interest.

16. The method of claim 1, wherein the messenger RNA is an RNA encoding a polypeptide of interest and wherein the reverse complement of the messenger RNA encoding the polypeptide of interest comprises a sequence complementary to all or a portion of an open reading frame of the messenger RNA encoding the polypeptide of interest.

17. A method of producing messenger RNA that comprises an open reading frame, the method comprising:

(a) forming a reaction mixture comprising a DNA template and nucleoside triphosphates (NTPs) including adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP), guanosine triphosphate (GTP), or an analog of each respective nucleoside, and a buffer; and

(b) incubating the reaction mixture to produce a composition comprising messenger RNA,

wherein the ratio of concentration of GTP to concentration of ATP is at least 2:1, the ratio of concentration of GTP to concentration of CTP is at least 4:1, and the ratio of concentration of GTP to concentration of UTP is at least 4:1.

18. The method of claim 17, wherein the ratio of concentration of GTP to concentration of ATP is 2:1, the ratio of concentration of GTP to concentration of CTP is 4:1, and the ratio of concentration of GTP to concentration of UTP is 4:1.