

Fluorophore RNA labeling by in vitro Transcription

- Linearize plasmid DNA; phenol/chloroform extract and ethanol precipitate, resuspend in dH₂O DEPC.
- Add the following to a microfuge tube on ice:

Fluorescein RNA Labeling Mix (Roche Cat. # 1685619)

Reagent	Quantity
Linearized plasmid DNA	1 µg
Fluorescein RNA Labeling Mix, 10× (or see below)	2 µl
10× Transcription Buffer (provided with RNA polymerases)	2 µl
dH ₂ O DEPC to a final volume of 18 µl	_ µl
RNA Polymerase (SP6, T7 or T3)	2 µl

or

Ribonucleoside Triphosphate Set + Fluorescein-UTP Labeling (if the Roche Labeling Mix is not used)

Reagent	Quantity
Linearized plasmid DNA	1 µg
Ribonucleoside Triphosphate Set, 10×	2 µl
Fluorescein-12-UTP (10 mM) – Final concentration 0.39 mM	0.7 µl
10× Transcription Buffer (provided with RNA polymerases)	2 µl
dH ₂ O DEPC to a final volume of 18 µl	_ µl
RNA Polymerase (SP6, T7 or T3)	2 µl

or

cy3 or cy5 Conjugated RNA Probe Synthesis (Perkin Elmer)

Reagent	Quantity
Linearized plasmid DNA	1 µg
Ribonucleoside Triphosphate Set, 10×	2 µl
cy3 or cy5 UTP (Perkin Elmer) (10 mM) – Final concentration 0.56 mM	1 µl
10× Transcription Buffer (provided with RNA polymerases)	2 µl
dH ₂ O DEPC to a final volume of 18 µl	_ µl
RNA Polymerase (SP6, T7 or T3)	2 µl

1. Mix and centrifuge briefly. Incubate 2 hours at 37° C.

2. Add on ice 2 μ l 0.2 M EDTA solution, pH 8.0 to stop the reaction.
3. The labeled RNA can be used directly for hybridization without further purification. Dilute to 80 μ l with dH₂O DEPC. Fluorophore-labeled RNA should not be extracted with phenol/chloroform. Store at -20°C.

Reagents

Ribonucleoside Triphosphate Set, 10 \times

Reagent	Quantity
ATP	2 μ l
CTP	2 μ l
GTP	2 μ l
UTP	1.3 μ l
dH ₂ O DEPC	12.7 μ l

Ribonucleoside Triphosphate Set - 100 mM lithium salt solutions (each UTP) (10 \times = 10 mM ATP,CTP,GTP; 6.5 mM UTP) – Use 2 μ l/reaction.

Reagent	Vendor	Catalog Number
Diethyl Pyrocarbonate (DEPC)	Sigma	D5758
Fluorescein-12-UTP	Roche	1427857
Fluorescein RNA Labeling Mix	Roche	1685619
Ribonucleoside Triphosphate Set - 100 mM lithium salt solutions	Roche	1277057
cy3-UTP (10mM)	Perkin Elmer	NEL582
cy5-UTP (10mM)	Perkin Elmer	NEL583