



Please adhere to good laboratory practice while following this guide, e.g. carry out the RNA production completely under laminar flow, wearing gloves.

Need support? Feel free to reach out to us at customer.service@quantoom.com

About this kit (mRNA)

This user guide covers the Ntensify[®] mano mRNA kit, which uses the Ntensify process (see Table 1 for performance summary). The kit supports up to 600µL of IVT reaction (or 24×25µL), yielding up to 100µg per 25µL reaction or 3mg for the full 600µL.

Table 1: Average performances of the Ntensify process related to process parameters and mRNA quality attributes.

IVT titer mg / mL	Purification yield %	RNA integrity post purification %
6.0	80.0	80.0

Pre-requisites not supplied in Ntensify mano

- **Equipment:** regular lab items, vortex, thermoshaker
- **Consumables:**
 - IVT: 0.5-1.5 mL RNase-free and DNA low binding micro-centrifuge tubes
 - Purification: 1.5 mL (Eppendorf[®] recommended) RNase-free and DNA low binding micro-centrifuge tubes
 - Final products: 1.5 mL RNase-free and DNA low binding micro-centrifuge tubes
- **Reagent:** absolute ethanol 99%
- **Linear DNA** at 0.5mg/mL with T7 promoter and a transcription start site “AG” for co-transcriptional capping (from 1 to 6kb).
- **Magnetic separation rack** - we recommend the Ntensify manoMAG (10001251)

Ntensify process overview

Table 2: Visualization of Ntensify mano components and process: integrated IVT and purification

	IVT							Purification				
	RNA transcription 75 min - 42.5°C - 800RPM			DNase 30 min - 42.5°C - 800RPM		Quenching 15 min - 42.5°C - 800RPM		RNA purification RT - 60 min				
	Control DNA	Mix 1a	UTP	Ψ-UTP	m1-Ψ-UTP	Mix 2	Mix 3	Mix 4	Mix 5	Mix 6	BB	NFW
Components	Nucleotides & cap analog					T7	IVT buffers	DNase	IVT block	Magnetic beads	Binding buffer	Nucleases-free water
Labels			U	P	M							
Storage box	-20°C							RT				
Volume (µL)	6	290	80	80	80	82	156	39	172	500	3000	4500

1. IVT step

Table 3: Reagent volumes for IVT reaction (µL)

		IVT							
		NFW	Mix 3	Mix 1a	U P M	Linear DNAs and/or Control DNA	Mix 2	Mix 4	Mix 5
IVT reaction volumes (µL)	25	2.87	5.00	10.00	2.50	2.00	2.62	1.25	5.00
	50	5.75	10.00	20.00	5.00	4.00	5.25	2.50	10.00
	100	11.50	20.00	40.00	10.00	8.00	10.50	5.00	20.00
	Your volume								

1. Set the thermoshaker at 42.5°C.
2. Thaw reagents ● Mix 1a, the uridine of your choice, ● Mix 3 and linear DNAs at RT (your DNA sample(s) and/or ● control DNA) ensure complete thawing of reagents.
3. Based on the instructions in Table 3, pipette the appropriate volumes of the following reagents in the specified order in the 0.5ml tubes mentioned in the consumables section:
 - NFW
 - Mix 3
 - Mix 1a + U or P or M
 - Linear DNAs
 - mix 2 (remove ● mix 2 from the freezer shortly prior to its usage). **Do not vortex!**
4. Vortex mix for 3–5 seconds and briefly spin down the tube before performing the incubation.
5. Incubate in the thermoshaker at 42.5°C, 800 rpm, for 75 minutes.
6. Remove from the freezer ● mix 4 shortly prior to its usage and add the required volume to the mix tube. **Do not vortex!**
7. Gently tilt the tube to homogenize it, then spin it down.
8. Incubate in the thermoshaker at 42.5°C, 800 rpm, for 30 minutes.
9. Add ● mix 5 to the mix tube and vortex mix for 3–5 seconds to homogenize.
10. Incubate in the thermoshaker at 42.5°C, 800 rpm, for 15 minutes.

Note: Transcribed RNAs can be stored at -80°C before proceeding to the purification.

2. Purification step

Table 4: Reagent volumes for purification

Purification volumes (μl)	IVT volumes (μl)	Mix 6 conditioning				Ethanol 80% preparation		Binding	Washing	Elution
		Mix 6	BB Volume 1	BB Volume 2	BB Volume 3	Absolute ethanol	NFW	1X	4X	2X
								Absolute ethanol	Ethanol 80% Volumes per wash	NFW Volumes per elution
25	25	16.7	33.4	50.0	16.7	180.0	45.0	8.4	50.0	50.0
50	50	33.4	66.7	100.0	33.4	360.0	90.0	16.7	100.0	100.0
100	100	66.7	133.4	200.0	66.7	720.0	180.0	33.4	200.0	200.0
Your volume										

● mix 6 conditioning prior to mRNA purification

- Vortex ● mix 6 until the obtention of homogenous bead suspension.
- Based on table 4, pipette the appropriate volume of ● mix 6 in a **clean** and **new** 1.5 mL Eppendorf® tube.
- Based on table 4, add volume 1 of ● binding buffer into the tube containing beads.
- Vortex tube 10 seconds vigorously (3000 rpm), then spin down the tube.
- Place tube on **Ntensify mano**MAG and allow bead magnetization for 2 minutes.
- Discard the supernatant using a micropipette.
- Based on table 4, add volume 2 of ● binding buffer into the tube containing beads.
- Vortex tube 10 seconds vigorously (3000 rpm), then spin down the tube.
- Place tube on **Ntensify mano**MAG and allow bead magnetization for 2 minutes.
- Discard the supernatant using a micropipette.
- Based on table 4, add volume 3 of ● binding buffer into the tube containing beads.
- Vortex tube 10 seconds vigorously (3000 rpm), then spin down the tube.

Ethanol 80% preparation

- Based on Table 4, pipette the appropriate volume of Absolute Ethanol in a clean and new Eppendorf® tube.
- Based on Table 4, add the appropriate volume of NFW into the tube containing the Absolute Ethanol.
- Vortex tube 10 seconds vigorously (3000 rpm), then spin down the tube.

The conditioned ● mix 6 is ready to use for the «binding» step

- Add the appropriate volume of IVT (refer to table 4; the excess can be used for analysis) to the conditioned ● mix 6 and vortex for 10 seconds vigorously (3000 rpm).
- Add **absolute ethanol** (not provided), vortex for 10 seconds vigorously (3000 rpm).
- Incubate in the thermoshaker at 1000 RPM for 1 minute at RT.
- Place the mix tube on **Ntensify mano**MAG magnet to allow the beads to separate from the supernatant (less than 20 seconds), and promptly discard the supernatant once it is free of beads.
- Repeat the following process from steps **a** and **b** for a total of four times:

WASHING

4x

- Add **ethanol 80%** (previously prepared) and vortex 10 seconds vigorously (3000 rpm).
- Place mix tube on **Ntensify mano**MAG magnet to allow the beads to separate from the supernatant (20 seconds), and promptly discard the supernatant once it is free of beads.

- Repeat the following steps from steps **a** to **c** for a total of two times:
- ELUTION

2x

 - Add ● **NFW**, vortex 10 seconds vigorously (3000 rpm), and incubate in the thermoshaker at 1000 rpm for 5 minutes at RT.
 - Place mix tube on the **Ntensify mano**MAG magnet to allow separation of beads from purified RNA (2 minutes).
 - Collect** the supernatant once it is free of beads.
- Pool the two elution fractions together in the same tube and homogenize the elution pool by gently agitating the tube.

Abbreviations

- IVT: *in vitro* transcription
- RT: room temperature
- NFW: nuclease-free water
- N/A: not applicable
- BB: binding buffer
- UTP: Uridine-5'-triphosphate
- Ψ-UTP: Pseudouridine-5'-triphosphate
- m1-Ψ-UTP: 1-Methylpseudouridine-5'-triphosphate

