Ntensify® mano

Manual

Version 1.1

For Research Use Only

This is the user guide for **Ntensify**® mano. For additional information, please visit Quantoom's website (quantoom.com).

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**

Pre-requisites not supplied in Ntensify mano

- **Equipment:** regular lab items, vortex, thermoshaker
- **Consumables:**
  - IVT: 0.5 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

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

Ntensify process overview

Table 1: Visualization of Ntensify mano components and process: integrated IVT and purification

	IVT	Purification
	RNA transcription 42.5°C 120 min	RNA purification RT 60 minutes

Components	Control DNA	Mix 1a	UTP	Ψ-UTP	m1-Ψ-UTP	Mix 2	Mix 3	Mix 4	Mix 5	Mix 6	BB	NFW
Labels	●	●	Ⓤ	Ⓟ	Ⓜ	●	●	●	●	●	●	●
Storage box	-20°C	-20°C	-20°C	-20°C	-20°C	-20°C	-20°C	-20°C	RT	RT	RT	RT
Volume (µL)	6	50	20	20	20	30	60	15	60	200	500	3000

1. IVT step

Table 2: Reagent volumes for IVT reaction (µL)

IVT reaction volumes (µl)	NFW	Mix 3	Mix 1a	Ⓤ Ⓟ Ⓜ	Linear DNAs and/or Control DNA	Mix 2	Mix 4	Mix 5
Your volume ____	____	____	____	____	____	____	____	____
50	17.76	10.00	10.00	3.00	4.00	5.26	2.50	8.30
100	35.50	20.00	20.00	6.00	8.00	10.50	5.00	16.60
200	71.00	40.00	40.00	12.00	16.00	21.00	10.00	33.20

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 2, 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 + Ⓤ or Ⓟ or Ⓜ
   - ● 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.

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2. Purification step

Table 3: Reagent volumes for purification

Purification volumes (µl)	IVT volumes (µl)	Binding 1X: Mix 6	Binding 1X: Absolute ethanol	Washing 4X: Absolute ethanol (Volumes per wash)	Washing 4X: NFW (Volumes per wash)	Elution 2X: NFW (Volumes per elution)
50	50	33.4	16.8	80.0	20.0	100.0
100	100	66.7	33.3	160.0	40.0	200.0
200	200	133.3	66.7	320.0	80.0	400.0

● mix 6 conditioning prior to mRNA purification

1. Vortex ● mix 6 **until** the obtention of homogenous bead suspension.
2. Based on table 3, pipette the appropriate volume of ● mix 6 in a **clean** and **new** 1.5 mL Eppendorf™ tube.
3. Place tube on **Ntensify** manoMAG and allow bead magnetization for 2 minutes.
4. Collect supernatant and measure its **exact** volume (SN_1)* using a micropipette.
5. Add SN_1 volume of ● binding buffer into the tube containing beads.
6. Vortex tube 10 seconds vigorously (3000 rpm), then spin down the tube.
7. Place tube on **Ntensify** manoMAG and allow bead magnetization for 2 minutes.
8. Collect supernatant and measure its exact volume (SN_2)* using a micropipette.
9. Add SN_2 volume of ● binding buffer into the tube containing beads.
   Caution: *measured volumes SN_1 and SN_2 of supernatant can be different.*
10. Vortex tube 10 seconds vigorously (3000 rpm), then spin down the tube.

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

11. Add the appropriate volume of IVT (refer to table 3; the excess can be used for analysis) to the conditioned ● mix 6 and vortex for 10 seconds vigorously (3000 rpm).
12. Add **absolute ethanol** 99% (not provided), vortex for 10 seconds vigorously (3000 rpm).
13. Incubate in the thermoshaker at 1000 RPM for 1 minute at RT.

* Collect the SN with larger volume and adjust the pipette volume until the liquid comes at the end of the tip

14. Place the mix tube on **Ntensify** manoMAG 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.
15. Repeat the following process from steps **a** to **c** for a total of four times:

WASHING **4x**

   a. Add **absolute ethanol** 99% and vortex 10 seconds vigorously (3000 rpm).
   b. Add ● **NFW** and vortex 10 seconds vigorously (3000 rpm).
   c. Place mix tube on **Ntensify** manoMAG magnet to allow the beads to separate from the supernatant (20 seconds), and promptly discard the supernatant once it is free of beads.

16. Repeat the following steps from steps **a** to **c** for a total of two times:

ELUTION **2x**

   a. Add ● **NFW**, vortex 10 seconds vigorously (3000 rpm), and incubate in the thermoshaker at 1000 rpm for 5 minutes at RT.
   b. Place mix tube on the **Ntensify** manoMAG magnet to allow separation of beads from purified RNA (2 minutes).
   c. **Collect** the supernatant once it is free of beads

17. Pool **both** elution fractions together in the same tube.

From small scale to large scale using the same process

Choose the solution adapted to your needs

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