



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

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About this kit (saRNA)

This user guide covers the **Ntensify[®]mano** saRNA kit, which uses the Ntensify process (see Table 1 for performance summary). The kit supports up to 400µL of IVT reaction (or 8×50µL), yielding up to 320µg per 50µL reaction or 2.5mg for the full 400µL.

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

IVT titer mg / mL	Purification yield %	RNA integrity post purification %
8	80	81.9

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 0.25mg/mL with T7 promoter and a transcription start site “AU” for co-transcriptional capping (from 8 to 12kb).
- **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 60 min - 42.5°C - 800RPM	DNase 10 min - 42.5°C - 800RPM	Quenching 15 min - 42.5°C - 800RPM	RNA purification RT - 60 min					
Viials	Control DNA sa	Mix 1b	Mix 2b	Mix 3b	Mix 4b	Mix 5b	Mix 6b	BBsa	NFW
Components	Nucleotides & cap analog	T7	IVT buffers	DNase	IVT block	Magnetic beads	Binding buffer sa	Nucleases-free water	
Labels									
Storage box	-20°C					RT			
Volume (µL)	11	242	74	106	53	92	368	3000	3000

1. IVT step

Table 3: Reagent volumes for IVT reaction (µL)

		IVT					
		Mix 3b	Mix 1b	DNA	Mix 2b	Mix 4b	Mix 5b
IVT reaction volumes (µL)	50	10.0	25.0	8.0	7.0	5.0	7.5
	100	20.0	50.0	16.0	14.0	10.0	15
	200	40.0	100.0	32.0	28.0	20.0	30
	Your volume	_____	_____	_____	_____	_____	_____

1. Set the thermoshaker at 42.5°C.
2. Thaw reagents **Mix 1b**, **Mix 3b** and linear DNAs at RT (your DNA sample(s) and/ or **control DNA sa**) 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:
 - Mix 3b**
 - Mix 1b**
 - Linear DNAs**
 - mix 2b** (remove **mix 2b** 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 60 minutes.
6. Remove from the freezer **mix 4b** 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 10 minutes.
9. Add **mix 5b** 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

		Mix 6b conditioning				Ethanol 80% preparation		Binding	Washing	Elution	
		Mix 6b	BBsa Volume 1	BBsa Volume 2	BBsa Volume 3	Absolute ethanol	NFW	1X	4X	2X	
								Absolute ethanol	Ethanol 80% Volumes per wash	NFW Volumes per elution	
Purification volumes (µl)	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
	200	200	133.4	266.8	400.0	133.4	1440.0	360.0	66.8	400.0	400.0
	Your volume										

● mix 6b conditioning prior to saRNA purification

- Vortex ● mix 6b until the obtention of homogenous bead suspension.
- Based on table 4, pipette the appropriate volume of ● mix 6b in a **clean** and **new** 1.5 mL Eppendorf® tube.
- Based on table 4, add volume 1 of ● Binding Buffer sa 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 sa 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 sa 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 6b 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 6b 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
- **BBsa**: binding buffer saRNA

