

Problems	Causes	Solutions
The RNA yield is lower than expected	Root-cause arising during IVT	
	One or several reagents were pipetted into the IVT reaction with the wrong volume.	Due to small volumes, pipette reagents with accuracy.
	During one or several incubations, the temperature(s) was/were inaccurate.	Ensure both incubations of the IVT step occur at 42.5°C.
	During one or several incubations, the incubation time(s) was/were inadequate.	The recommended incubation times: for IVT: 75 min ± 5 for DNase: 30 min ± 5
	The IVT reaction was agitated at an inadequate speed (rpm).	Ensure the reaction is agitated at 800rpm after adding mix 2 and again at 800rpm after adding mix 4.
	The DNA templates do not conform all required sequence pre-requisites	Ensure to follow the minimal requirements for DNA templates: - templates: either linearized DNA, or PCR, or synthetic DNA oligo-nucleotides - contaminant-free sample - recommended length between 1 and 10 kb - contains all of the following: * T7 promoter region * a AG initiation sequence upstream of the ORF (Open Reading Frame) * poly(A)-tail * terminator
	Mixes 2 and 4 were vortexed	Mix 2 nor 4 should not be vortexed.
	One or several reagent(s) are degraded.	Ensure all reagents are stored at the recommended temperatures. These can be found in both the bottle label and the kit box. Some reagents are at a room temperature, some others at -20°C. Also, ensure that all reagents are not expired.

The RNA yield is lower than expected	The quantity of DNA templates is inadequate for the reaction.	It is recommended to use the concentration of 0.5µg/µL for the DNA templates. - if your concentration is higher, dilute DNA templates with RNase-free water to 0.5µg/µL, measure sample concentration, and then start the IVT reaction - If your concentration is lower, option 1: use the table 2 in Ntensify[™] mano protocol to adapt directly in the IVT reaction the volumes for both DNA templates and water. The goal is to have 40µg of DNA templates for each 1mL of IVT reaction. option 2: concentrate your DNA using a membrane, and elute it in the correct volume for 0.5µg/µL.
	Root-cause arising during DSP	
	The volume of magnetic beads was insufficient.	Ensure during the mix 6 conditioning that the beads are resuspended in the exact volume of conditioning buffer, as this is important for the purification step.
	Beads were (partially) lost when pipetting up after the binding and/or washing steps.	Ensure all beads are magnetized before pipeting up gently and slowly the supernatant. Wait for 25 seconds for the magnetization of all beads.
	During purification, the magnet used is inadequate.	Request a manoMAG magnet from Quantoom for easy and complete bead magnetization and perfect positioning of tubes.
	The incubation time during the elution step was too short.	For the elution step, incubate the beads in NFW (nuclease-free water) for 15 minutes.
	The mix 6 was not conditioned before producing RNAs using Ntensify [™] mano.	It is highly recommended to condition the mix 6 before producing RNAs with the Ntensify [™] mano
	There was an RNase contamination	To avoid RNase contamination, use RNase-free disposables & water, appropriate PPEs and work under airflow.
The RNA yield is lower than expected	One or several reagent(s) are degraded.	Ensure all reagents are stored at the recommended temperatures. These can be found on both the bottle label and the kit box. Some reagents are at a room temperature, some other at -20°C. Also, ensure that all reagents are not expired.

I obtained RNA with the control DNA but not with my DNA template	The DNA template does not meet the DNA requirements.	Ensure to follow the minimal requirements for DNA templates: - templates: either linearized DNA, or PCR, or synthetic DNA oligo-nucleotides - contaminant-free sample - recommended length between 1 and 10 kb - contains all of the following: * T7 promoter region * a AG initiation sequence upstream of the ORF (Open Reading Frame) * poly(A)-tail * terminator
	One or several reagents were pipetted into the reaction with the wrong volume.	Due to small volumes, pipette reagents with accuracy.
	The quantity of DNA templates is inadequate for the reaction.	It is recommended to use the concentration of 0.5µg/µL for the DNA templates. - if your concentration is higher, dilute DNA templates with RNase-free water to 0.54ng/µL, measure sample with Nanodrop, and then start the IVT reaction - If your concentration is lower, use the table 2 in Ntensify™ mano protocol to adapt directly in the IVT reaction the volumes for both DNA templates and water. The goal is to have 40µg of DNA templates for each 1mL of IVT reaction.
Integrity	During one or several incubations, the incubation time(s) was/were inadequate.	The recommended incubation times are: for IVT: 75 min ± 5 for DNase: 30 min ± 5
	The binding time on beads was too long.	After adding the absolute ethanol, incubate on magnetic beads for 1 min only.
	The mix 6 was not conditioned before producing RNAs using Ntensify™ mano.	It is highly recommended to condition the mix 6 before producing RNAs with the Ntensify™ mano