



**Figure 1.**

- Size exclusion chromatography (S200, Cytiva) of RT domain after heparin chromatography, tag cleavage, and tag removal shows a single monodisperse species with no evidence of contaminating nucleic acids.
- SDS-PAGE of SEC fractions shows isolated RT with high purity suitable for structural and mechanistic studies.
- Reverse transcriptase activity assay (Roche, polyA template) shows high specific activity of RT, increasing during purification.
- Representative hole magnification screening image of RT-ternary complex. A combination of R1.2/1.3 Quantifoil 300 mesh (pictured) and R0.6/1 200 mesh grids were imaged on Titan Krios with Gatan K2.
- Representative high magnification image of RT-ternary complex show discrete, spread monodisperse particles proximately 100 angstroms in diameter, consistent with expectations from homologous structures and AlphaFold.