



Characterization of a new reference material for human cardiac troponin: GBW09336

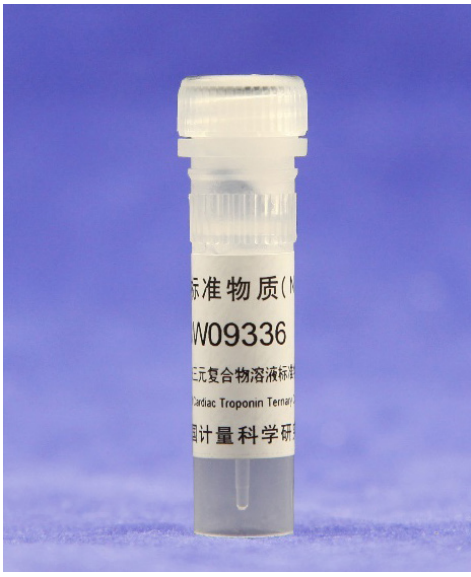
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INTRODUCTION

The immunochemical detection of cardiac troponin I (cTnI) or T (cTnT) is an essential method for the diagnosis of myocardial infarction. Reliable determination of biomarker concentration by immunochemical assays requires the use of a well-characterized and traceable protein standard. In this study we present a recombinant ternary human troponin complex (recITC) as a homogeneous, reproducible, and scalable alternative to a tissue-derived protein (natITC).



MATERIALS AND METHODS

RecITC was formed from the purified troponins expressed in *E. coli*. The material was characterized using SDS-PAGE, Western blotting (WB), reversed-phase HPLC, mass spectrometry (MS), and peptide mapping. A structural comparison with native human troponin was performed using hydrogen-deuterium exchange mass spectrometry (HDX-MS). The immunoreactivity of recITC was assessed using five monoclonal antibody pairs targeting diverse epitopes. Value assignment was performed using two independent isotope dilution mass spectrometry (IDMS) methods with SI traceability. The homogeneity and stability of recITC were evaluated according to ISO 33405:2024.

RESULTS

Purity and structural integrity of recITC

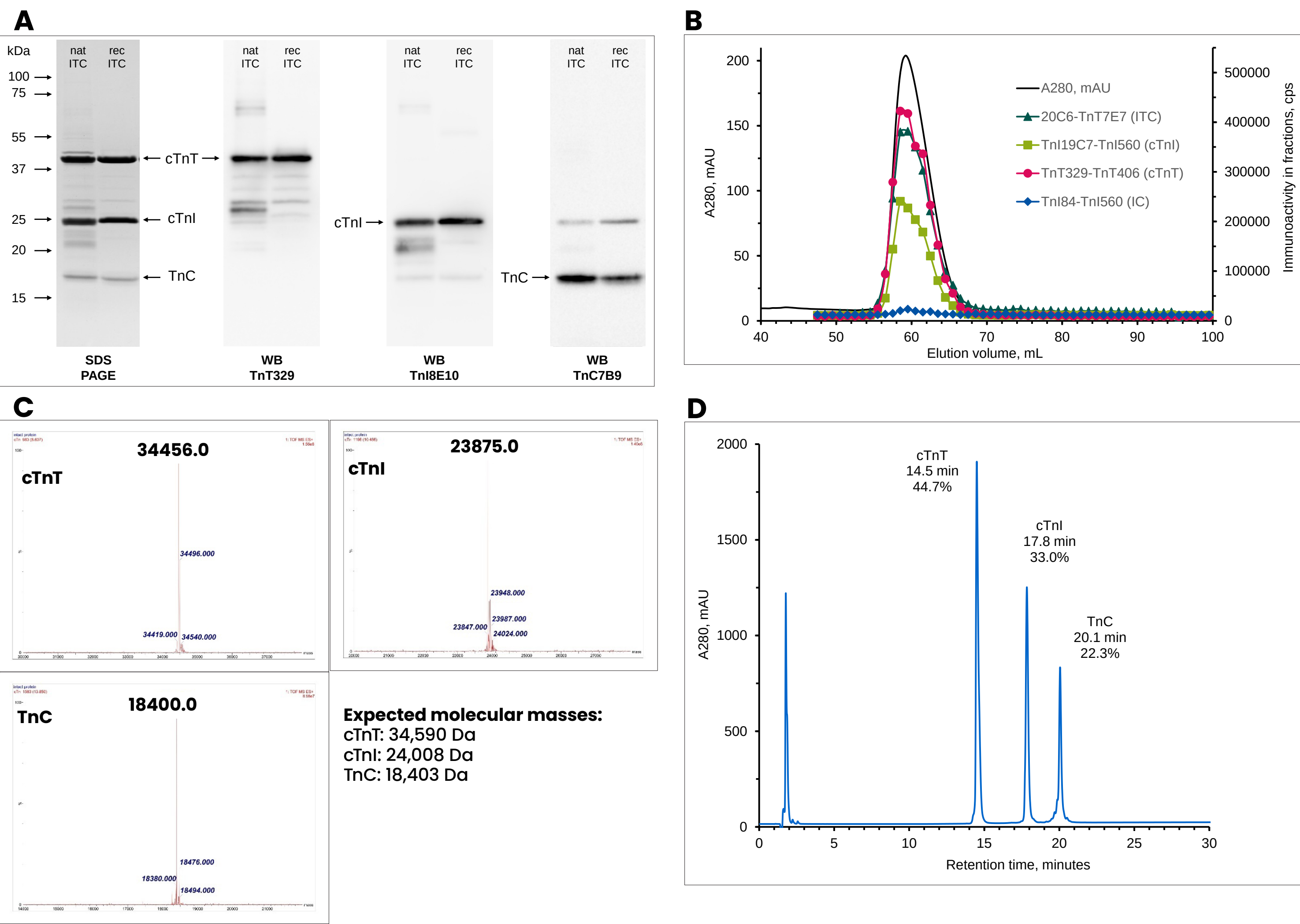


Figure 1. WB, gel filtration, reversed-phase chromatography and MS analyses of recITC. A – Comparison of protein composition of natITC and recITC complexes. SDS-PAGE (left, 0.5 µg of cTnI per lane) and WB analysis (center and right, 0.25 µg of cTnI per lane) of ITC complexes. B – Gel filtration of recITC on a Superdex 200 16/60 column. C – UPLC-Q-TOF analysis of recITC. D – Reversed-phase liquid chromatography of recITC (ACQUITY UPLC Protein BEH C4 Column).

The recITC preparation consisted only of cTnT, cTnI and TnC, whereas the natITC preparation comprised minor protein impurities with a relative molecular weight of approximately 45 kDa. FPLC gel filtration analysis further confirmed that the recITC preparation contained only the ternary complex, with no detectable free troponins or binary IC complex.

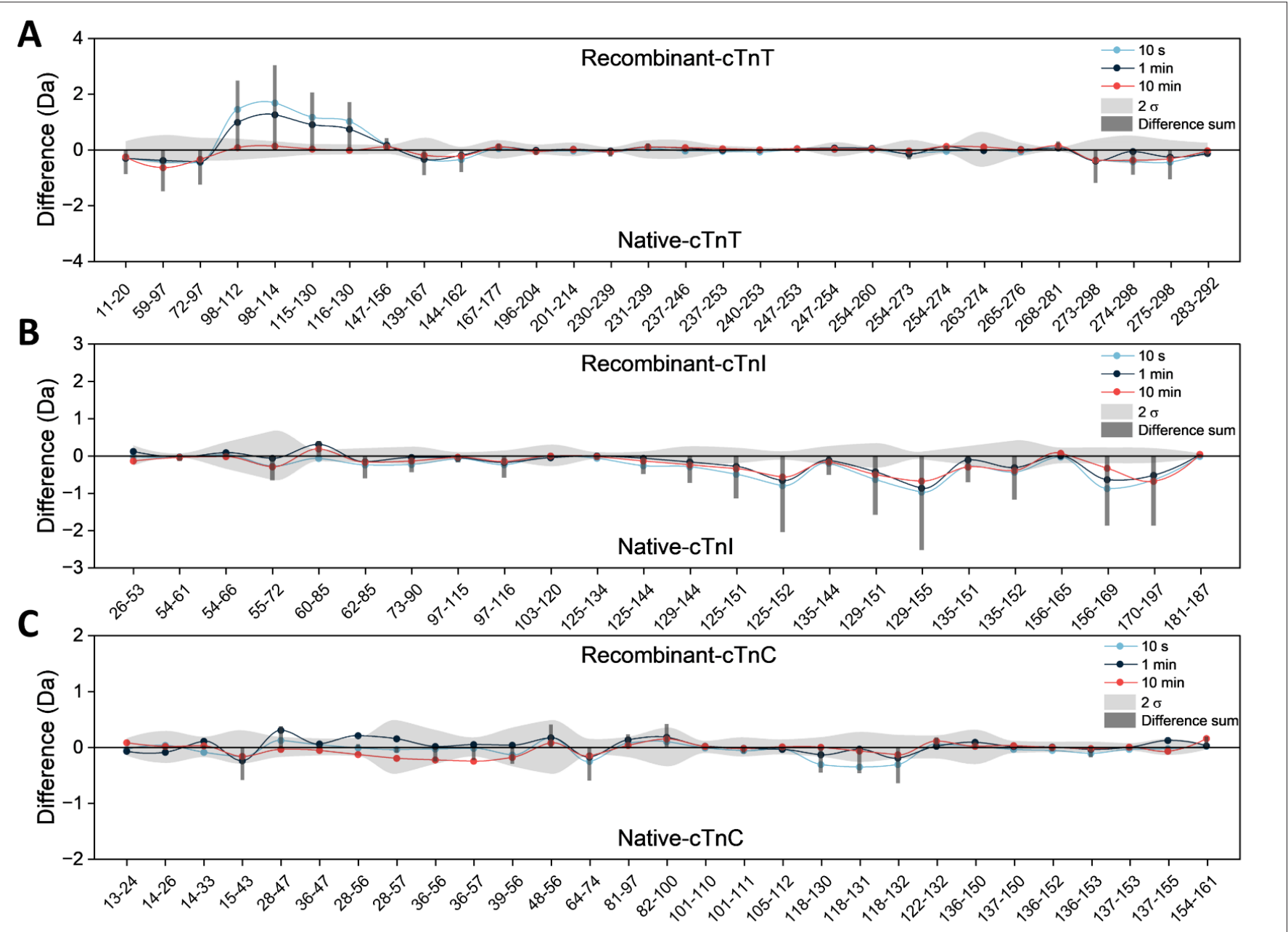


Figure 2. HDX-MS analysis of recombinant and native cTn subunits. A – Recombinant cTnT vs. native cTnT. B – Recombinant cTnI vs. native cTnI. C – Recombinant cTnC vs. native cTnC at different time points (10 s, 1 min, 10 min). The x-axis represents the amino acid sequence positions of each subunit, and the y-axis represents the hydrogen-deuterium exchange differences (in Da) between the two forms. Different colors indicate hydrogen-deuterium exchange data at different time points; the light gray areas representing statistical error (2σ) and the 95% confidence interval, while the dark gray bars represent the cumulative differences across all three time points.

HDX-MS analysis of recombinant and native cTn subunits showed only subtle structural differences that do not affect the overall structure and are not expected to compromise the utility of recombinant cTn as a reference material.

RESULTS

Immunochemical activity of recITC

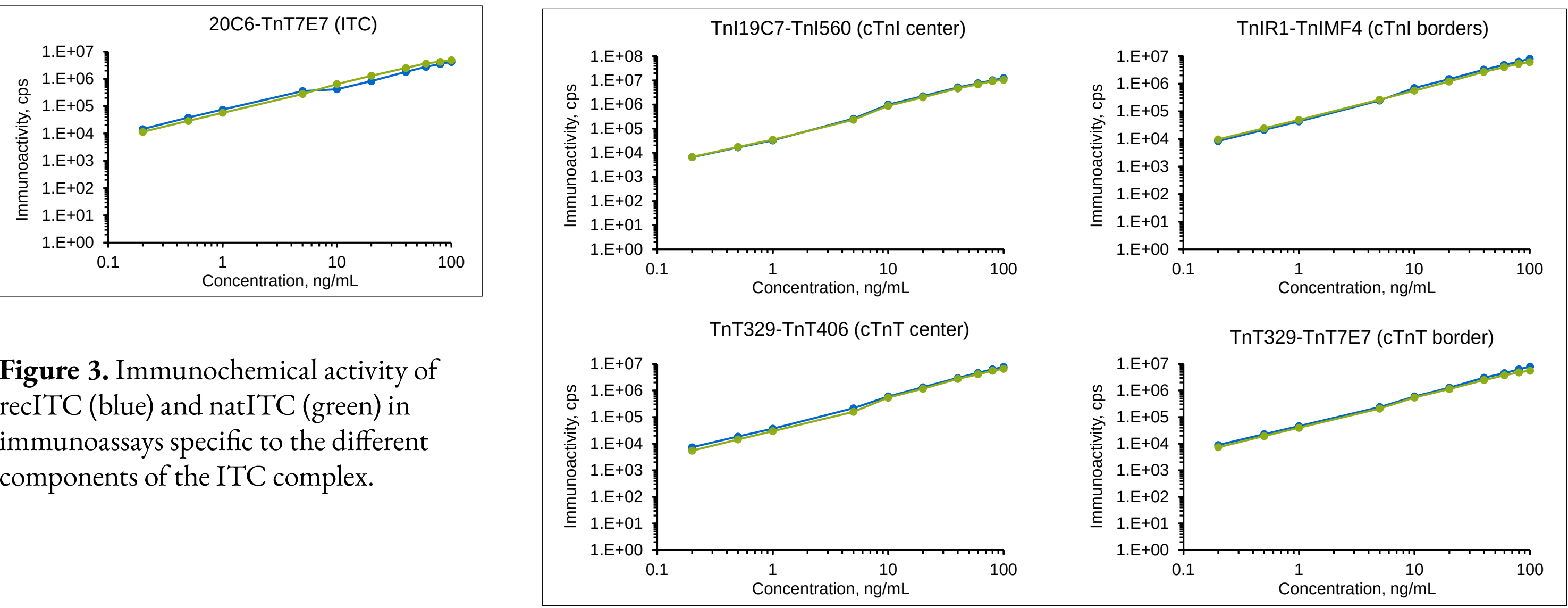


Figure 3. Immunochemical activity of recITC (blue) and natITC (green) in immunoassays specific to the different components of the ITC complex.

RecITC and natITC showed comparable immunoreactivity in all of the tested assays.

Stability of recITC

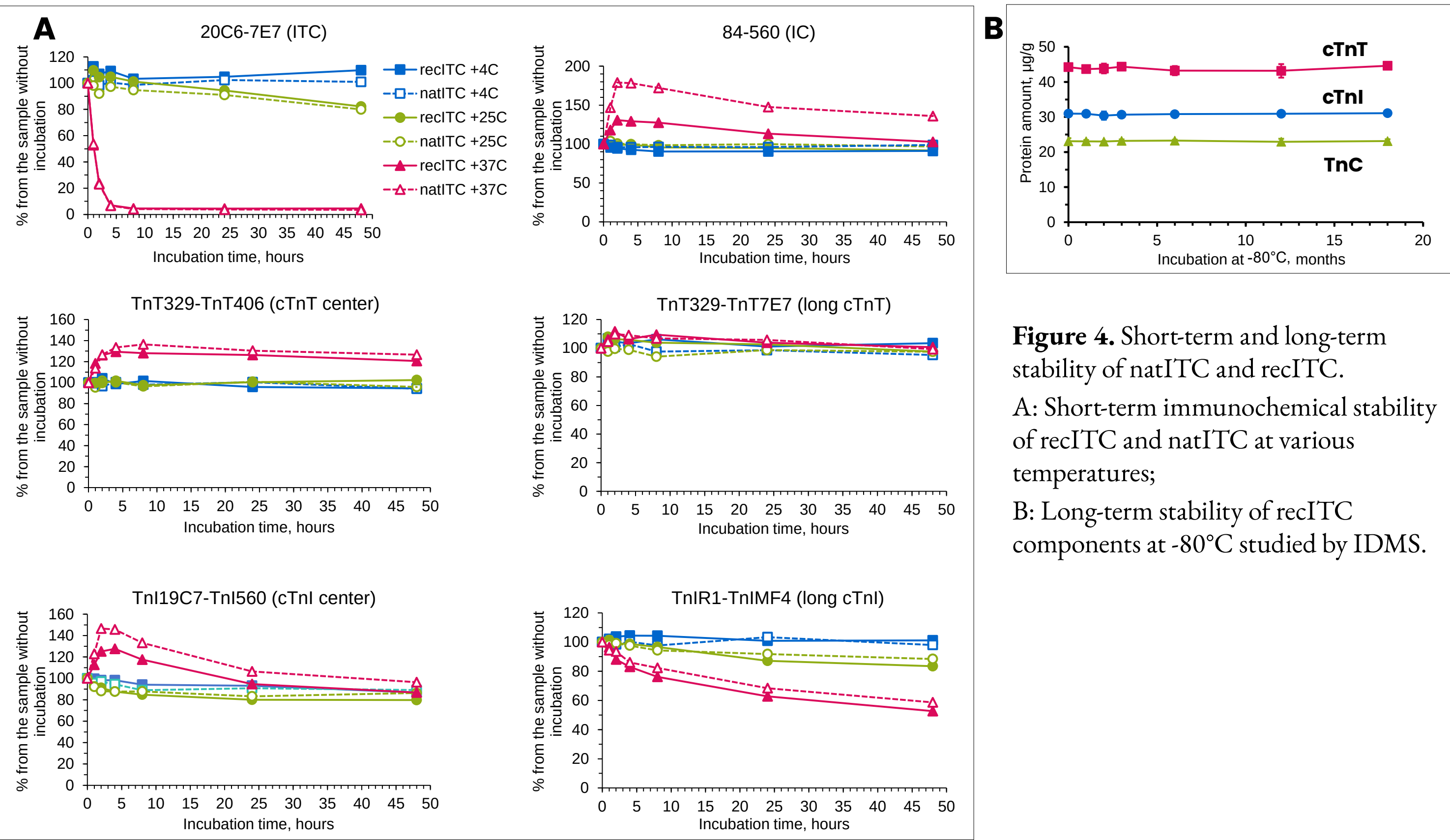


Figure 4. Short-term and long-term stability of natITC and recITC. A: Short-term immunochemical stability of recITC and natITC at various temperatures; B: Long-term stability of recITC components at -80°C studied by IDMS.

Both recITC and natITC were stable during the short-term incubation at +4°C and +25°C, but rapidly dissociated at +37°C.

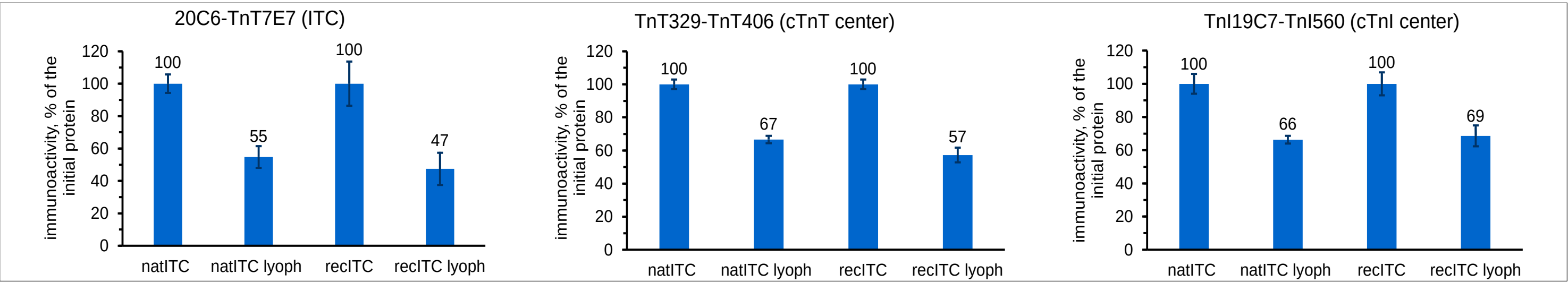


Figure 5. Lyophilization of natITC and recITC.

Lyophilization of both recITC and natITC led to a considerable decrease in the immunoactivity of all troponins; therefore, lyophilization is not recommended.

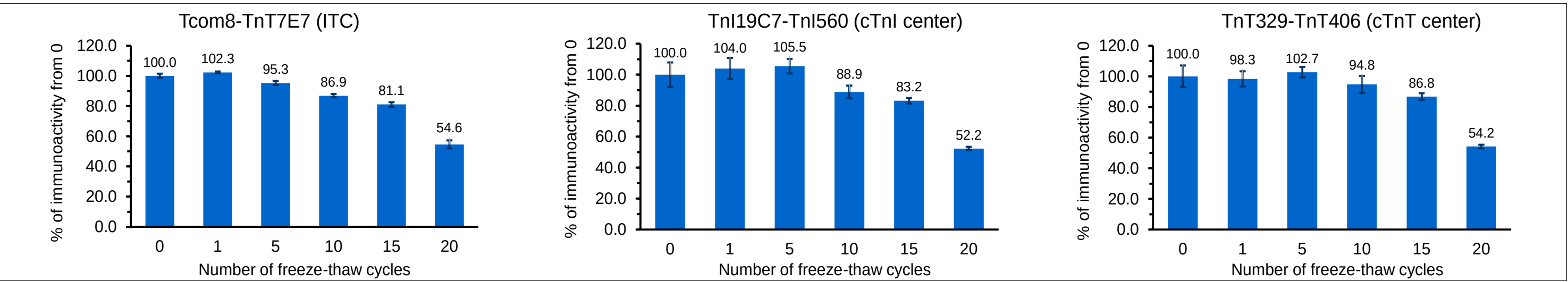


Figure 6. Freeze-thawing of recITC.

RecITC remained stable for up to five freeze-thaw cycles but began to lose immunoactivity thereafter; so repeated freeze-thawing is not recommended.

The certified values of GBW09336 are:

cTnI: (30.5 ± 1.4) µg/g (*k* = 2)

cTnT: (44.1 ± 2.2) µg/g (*k* = 2)

TnC: (23.5 ± 1.4) µg/g (*k* = 2)

CONCLUSIONS

GBW09336 is a sustainable, homogeneous, and stable reference material that provides SI-traceable certified values for all three subunits — cTnI, cTnT, and TnC.