Jul 07, 2025

Isothermal Titration Calorimetry (ITC)

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlkr7×6v5r/v1

Dieter Waschbuesch[1], Amir Khan[2]

[1]School ofBiochemistry and Immunology, Trinity College Dublin; [2]Trinity College Dublin

AKhanLab

Dieter Waschbuesch
School ofBiochemistry and Immunology, Trinity College Dublin

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlkr7x6v5r/v1

Protocol Citation: Dieter Waschbuesch, Amir Khan 2025. Isothermal Titration Calorimetry (ITC). **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkr7x6v5r/v1

Protocol status: Working
We use this protocol and it's working

Created: June 26, 2025

Last Modified: July 07, 2025

Protocol Integer ID: 221125

Keywords: isothermal titration calorimetry, peptide, itc, enthalpy, thermodynamic parameters such as enthalpy, quantitative data on affinity, protein, interacting protein, underlying thermodynamic parameter

Funders Acknowledgements:
Research Ireland
Grant ID: 20/FFP-A/8446

Abstract

This protocol describes in general our laboratory's way how to perform ITC between two interacting proteins or a protein and a peptide. It is a useful method to get quantitative data on affinity and the underlying thermodynamic parameters such as enthalpy.

Materials

Consumables

- 1.5 ml reaction tubes
- 0.5 ml PCR tubes
- Dialysis tube 12 kDa MWCO (Sigma, D9777)
- Pur-A-Lyzer™ Midi Dialysis tubes 1 kDa MWCO (Sigma, PURD10005-1KT) for peptides
- Millex -GP Syringe filters (Merck, SLGP033RS)

Reagents

- Purified proteins/peptides (protein A, protein/peptide B)
- Dialysis Buffer (20 mM Tris pH 8.0 at 4ºC, 150 mM NaCl, 1 mM DTT)

Note

Some proteins require the presence of additional co-factors, e.g. Rab GTPases require the presence of Mg^{2+}, which is added to the buffer in case an ITC experiment involves a Rab-GTPase. Different buffers also work, it is just important that the two interactors are dialysed into the same buffer.

Equipment

- Calorimeter ITC 2000 (MicroCal)
- 1 l laboratory beaker
- PC with the MicroCal ITC200 software (version 1.30, Malvern) and Origin 7 (OriginLab Corporation)
- Tabletop microcentrifuge (VWR MicroStar 12)

Protocol

1 Dialyse the proteins/peptide against dialysis buffer. For proteins, conventional dialysis tube is used, for small peptides the Pur-A-lyzer 1 kDa MWCO is used according to the manufacturer's instructions. Stir using a magnetic stirrer at 100-200 rpm (gentle in other words). Dialysis time should be at least 2 hours, if the buffer mismatch is greater, longer times are recommended (e.g. overnight at 4°C).

Note

To avoid buffer mismatch, the two proteins need to be dialyzed against the dialysis buffer in the same beaker.

2 Determine noise of the ITC instrument by titrating water into water with standard 'Noise' injection parameters from MicroCal (temperature, spin, injection volumes, etc) . A noise level <1.5 microCal/sec is deemed acceptable and the experiment will likely succeed.

3 Determine the concentration of the proteins or peptide and adjust volumes to the desired concentration (e.g. 500 µl of 40 µM protein A for the cell and 200 µl of 400 µM protein/peptide B).

Note

Concentrate proteins higher than the desired concentration. Filter some dialysis buffer to dilute proteins to the desired concentration.
A good starting point is for titrations is 40 µM of protein A in the cell of the instrument and 400 µM protein/peptide B in the syringe to titrate into the cell. The exact individual parameters need to be determined in the course of conducting the experiments.

4 Directly before the experiment, centrifuge proteins/peptide for 3-5 minutes at 12,300 x g.

Note

It can be beneficial to de-gas the protein sample for the cell before that spin.

5 Carefully fill the syringe to titrate into the cell with protein/peptide B (400 µM). About 40 µl is required for syringe, but practically, 80 µl minimum is required to insure filling.

6 Load ~350 µl protein A (40 µM) with a Hamilton syringe and inject smoothly into the sample cell of ITC200 (200 µl) to insure complete fill. Avoid bubbles.

7 Place injector into sample cell and run the MicroCal ITC200 software. Check the instrument and run settings:

- Reference power: 5 µcal/sec
- Temperature: 20°C
- Stir speed: 750 rpm
- Initial delay: 60 sec
- injection spacing: 180 sec
- Injection parameters: 22* injections, 2.0* µl each, 4* second duration, spaced by 180 sec. Initial inject was 0.5 µL with a duration of 1 sec.

Note

Parameters marked with an * are adjusted individually for the different protein/peptide combinations.

8 Run protocol in the software.

9 Analyse the data in Origin with the manufacturers data analysis module.