Optimize your HTRF cell signaling assays on tissues.

This technical note gives precise guidelines for tissue lysate preparation, assay optimization, data analysis, and then interpretation, guaranteeing you will get accurate and interpretable results.

Abstract

HTRF® Cell Signaling assays allow rapid and reliable measurement of the phosphorylation and the expression level of key signaling proteins in cell lysates. These assays are also suitable for tissue sample analysis to evaluate the *in vivo* efficacy of compounds on preclinical models. However, the proper preparation of lysates and working solutions using the right reagents is crucial to ensure you obtain the best results. Moreover, it is essential to include the appropriate controls to analyze and interpret the results correctly.

This technical note provides procedures and guidelines to optimize your HTRF Cell Signaling assays on tissues, with a focus on sample preparation and data analysis. Please note that the procedure for tissue lysis and sample preparation is also suitable for western-blot experiments.

Reagents and devices

- **HTRF cell signaling kit** including stock solutions of lysis buffer (4X), blocking reagent (100X) and detection reagents
- **Phosphate Buffered Saline (PBS)**
- **Protease inhibitor cocktail, EDTA-free** (e.g Roche #11836170001)
- **Tissue homogenizer** (e.g. GentleMACS™Dissociator, Miltenyi Biotec)

Buffer preparation

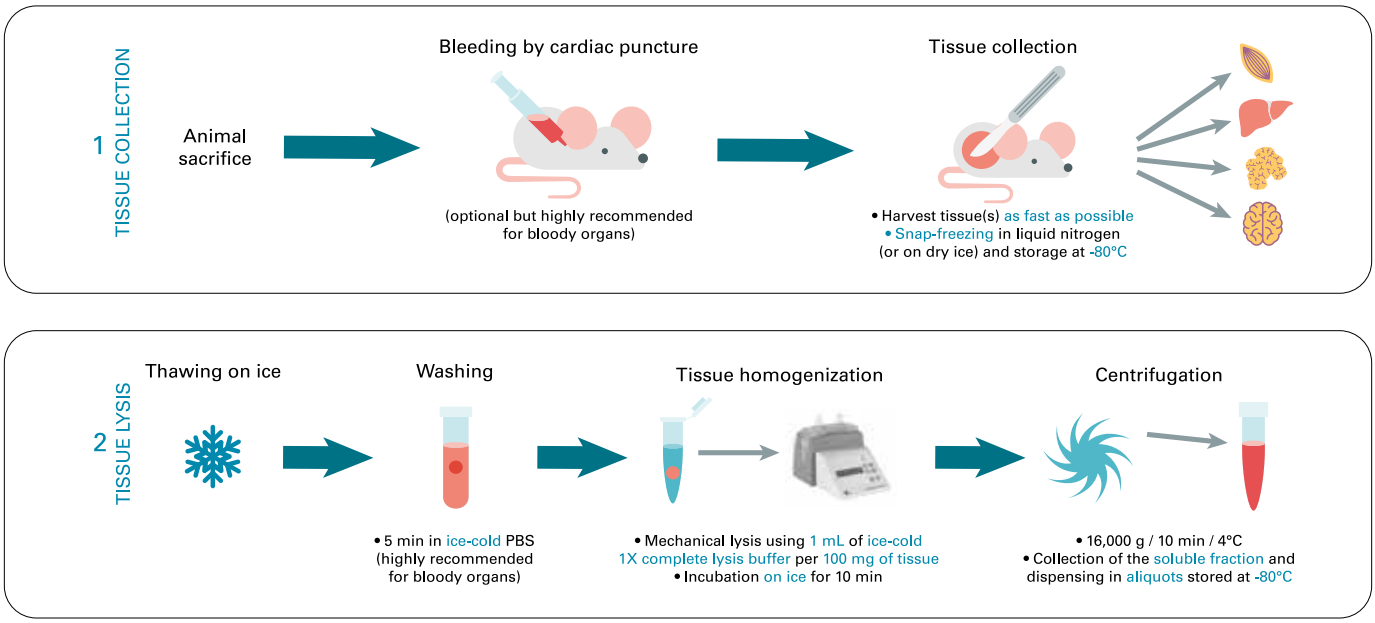
For tissue lysis:

Prepare **ice-cold 1X complete lysis buffer**: dilute lysis buffer stock solution with distilled water and complement with the **blocking reagent** and **protease inhibitors**. Place PBS on **ice**.

For sample preparation:

Prepare **ice-cold 1X supplemented lysis buffer**: prepare 1X lysis buffer in distilled water and supplement with the **blocking reagent**.

Procedure workflow for tissue collection and lysis

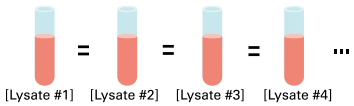


Sample preparation

1. **Determine protein concentration** (e.g. using the QuantiPro BCA Assay kit, SIGMA).

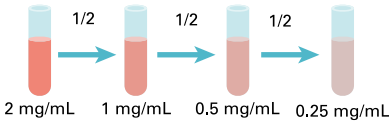
   HTRF lysis buffers do not contain any carrier proteins and thus are suitable for total protein quantification.

2. **Adjust sample concentration:**
   Thaw lysates **on ice** and adjust to the **same initial protein concentration** (e.g. 2 mg/mL) in **ice-cold 1X supplemented lysis buffer**. This step is mandatory to be able to compare results between different groups of samples (e.g. control vs. treated).



NB: Keep tissue samples **on ice** until they are dispensed into the HTRF detection microplate (#66PL96).

3. **Determine the optimal working solution:**
   Before running the first HTRF analysis, you must determine which lysate dilution ensures that you work within the linear range of the HTRF assay(s). Each lysate is therefore serially diluted in **ice-cold 1X supplemented lysis buffer**, and several dilutions are tested.



HTRF controls

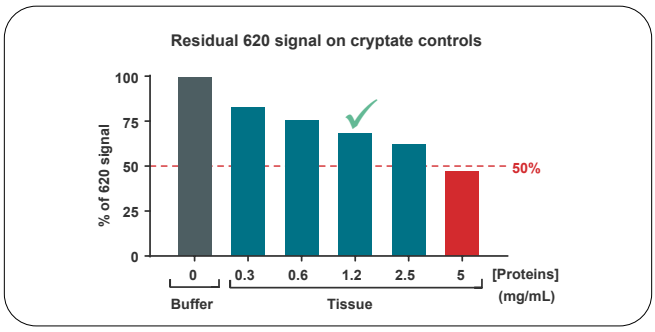
- For HTRF detection reagent preparation, please follow kit package insert's instructions. The dispensing step must include:
- **Sample:** 16 µL of each lysate dilution + 2 µL d2-antibody + 2 µL cryptate-antibody.
- **Cryptate tissue control:** 16 µL of each lysate dilution + 2 µL detection buffer + 2 µL cryptate-antibody.
- **Cryptate buffer control:** 16 µL 1X supplemented lysis buffer + 2 µL detection buffer + 2 µL cryptate-antibody.

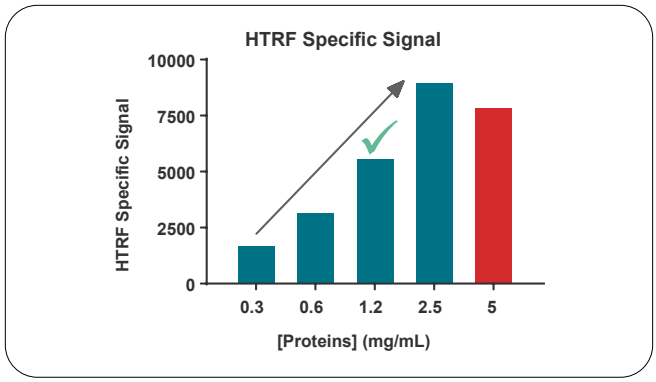
Data manipulation

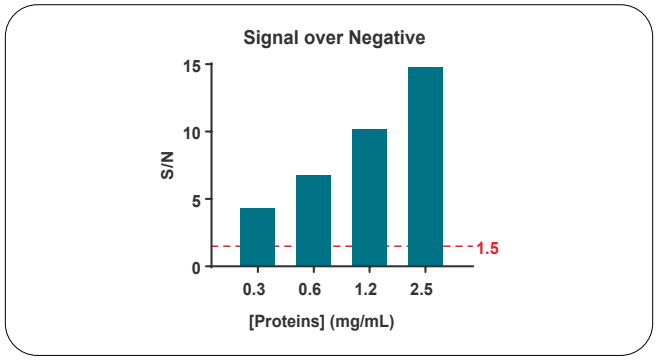
- **Residual 620 signal (%): must be > 50%**
  (620 signal $_{sample}$ / 620 signal $_{cryptate\ buffer\ control}$) x 100
- **HTRF Ratio:** (665 signal / 620 signal) x 10,000
- **HTRF Specific Signal: must be linear between two sample dilutions**
  HTRF Ratio $_{sample}$ - HTRF Ratio $_{cryptate\ tissue\ control}$
- **Signal over Negative (S/N): must be > 1.5**
  HTRF Ratio $_{sample}$ / HTRF Ratio $_{cryptate\ tissue\ control}$

Case study: optimization of advanced phospho-ERK assay on a mouse liver lysate

Analysis of a mouse liver lysate using the HTRF Advanced ERK phospho-T202 /Y204 kit (64AERPEG):







Samples containing between 0.3 and 2.5 mg/mL of proteins (blue bars) meet all requirements in terms of residual 620 signal and S/N. However, based on the analysis of the HTRF Specific Signal, the concentration of 1.2 mg/mL (green mark) was selected to ensure detecting correctly positive or negative signal modulations induced by drugs.

Conclusion

This technical note is a valuable complement to the HTRF Cell Signaling kit protocols for analyzing intracellular proteins in tissue lysates. It gives precise guidelines for tissue lysate preparation, assay optimization, data analysis, and then interpretation, guaranteeing you will get accurate and interpretable results.

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