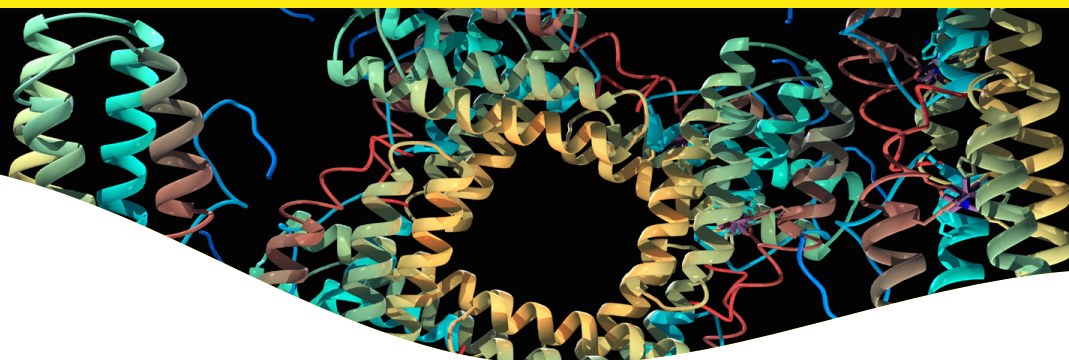




# LANCE protein:protein interaction quick start guide.



## Introduction

This quick start guide will take you through the first of three easy steps to develop a protein:protein interaction (PPI) assay utilizing LANCE® TR-FRET technology. For more information, including details on further optimization experiments, please refer to our full LANCE PPI assay development guide.

## Materials

- Recombinant Human GST-Protein X
- Recombinant Human His6-Protein Y
- LANCE Eu-W1024 labeled anti-GST antibody (Revvity Cat. # AD0252)
- LANCE Eu-W1024 Anti-6xHis (Revvity Cat. # AD0110)
- LANCE *Ultra ULight*<sup>™</sup>-anti-GST (Revvity Cat. # TRF0104-D)
- LANCE *Ultra ULight* -anti-6xHis (Revvity Cat. # TRF0105-M)
- OptiPlate-384, White Opaque 384-well Microplate (Revvity Cat. # 6007290)

Lance europium donor reagents	ULight and surelight APC acceptor reagents	
Eu-anti-c-myc	ULight-anti-c-myc	APC-anti-FLAG
Eu-anti-FITC	ULight-anti-FITC	APC-anti-GST
Eu-anti-FLAG	ULight-anti-FLAG	APC-anti-6X His
Eu-anti-GST	ULight-anti-GST	APC-anti-mouse IgG
Eu-anti-HA	ULight-anti-6X His	APC-anti-rabbit IgG
Eu-anti-6X His	ULight-anti-human IgG	APC-streptavidin
Eu-anti-human IgG	ULight-anti-mouse IgG	
Eu-anti-mouse IgG	ULight-anti-rabbit IgG	
Eu-anti-rabbit IgG	ULight-Protein A	
Eu-Protein G	ULight-Streptavidin	
Eu-Streptavidin		

## LANCE TR-FRET toolbox reagents

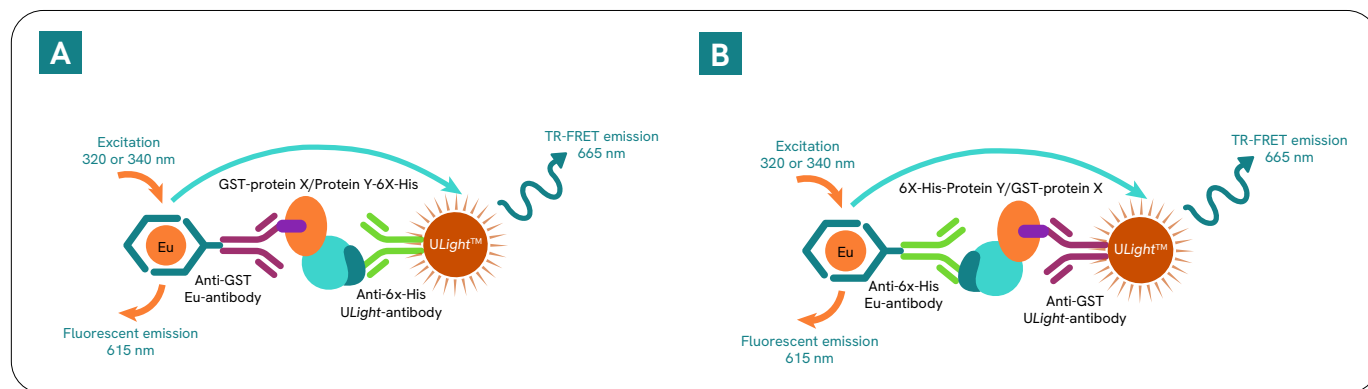


Figure 1: Illustration of a LANCE protein: protein interaction assay, using anti-GST Europium W1024 Chelate, Anti-6X-His ULight conjugate, 6X-His-Protein Y and GST-Protein (panel A). Reverse configuration is shown in panel B.

This first experiment will show you if your particular binding partners will work together in a TR-FRET PPI assay. It will also let you determine 1) the best donor and acceptor configuration, 2) the optimal concentrations of your binding partners and 3) the optimal incubation time of your assay. Figure 1 shows two possible configurations for capturing a complex of a GST-tagged protein and a His-Tagged protein in a PPI assay. The initial experiment involves a cross-titration of various concentrations of each protein. Figure 2 shows a representative plate map for a single replicate of one configuration. The optimal incubation time is determined by re-reading the assay plate at the time intervals listed in the experimental flow chart in Figure 3.

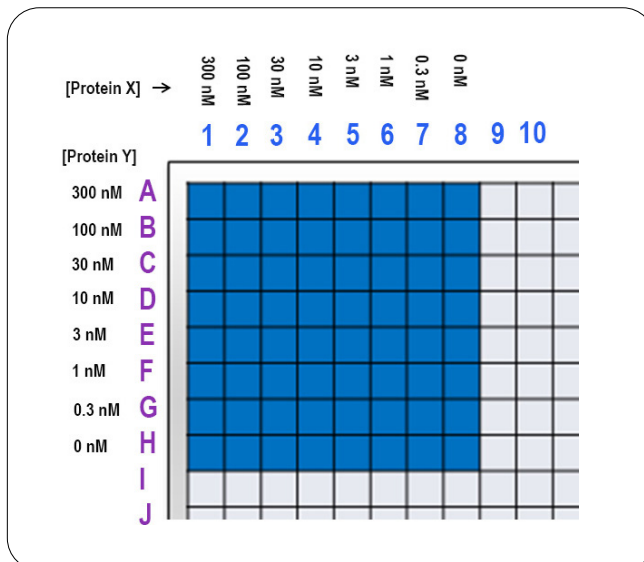


Figure 2: Plate layout utilized for one replicate of the 2D titration in Experiment #1, using 1 configuration of donor and acceptor reagents. Replicates of configuration 1 and the same concentrations of proteins using the alternate configuration 2 can be tested on the same plate.

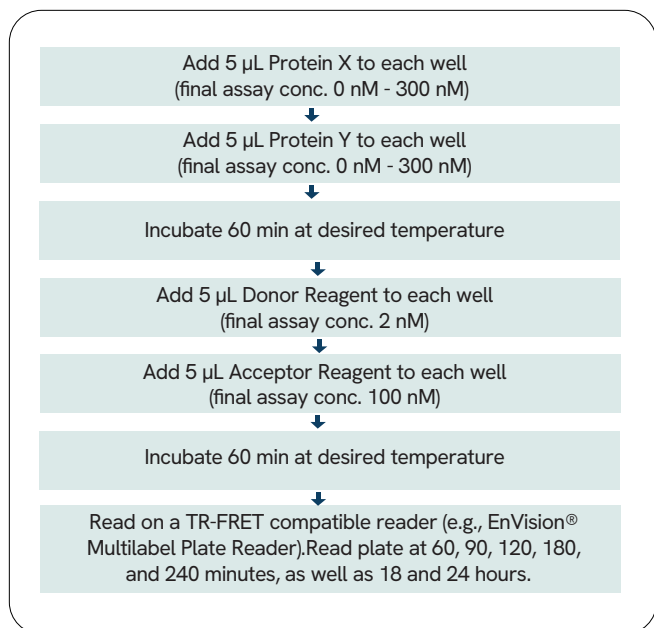
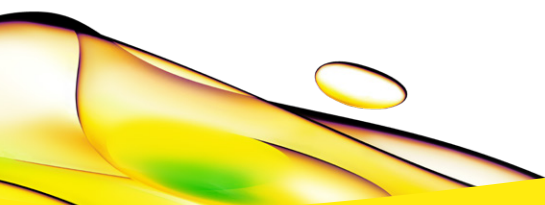


Figure 3: Protocol used for Experiment #1. Final assay volume is 20 µL, therefore each reagent is made up at 4X of the final concentration.

## Next steps for assay optimization

- Concentration-response of vehicle (e.g. DMSO), using optimal conditions from Experiment #1, at different concentrations of donor and acceptor reagents
- Concentration-response of inhibitors, using optimal conditions from Experiments #1 and #2, at multiple temperatures of incubation (if applicable), and with different orders of addition of reagents (if applicable)
- Time-course experiment (prior to addition of detection reagents)



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