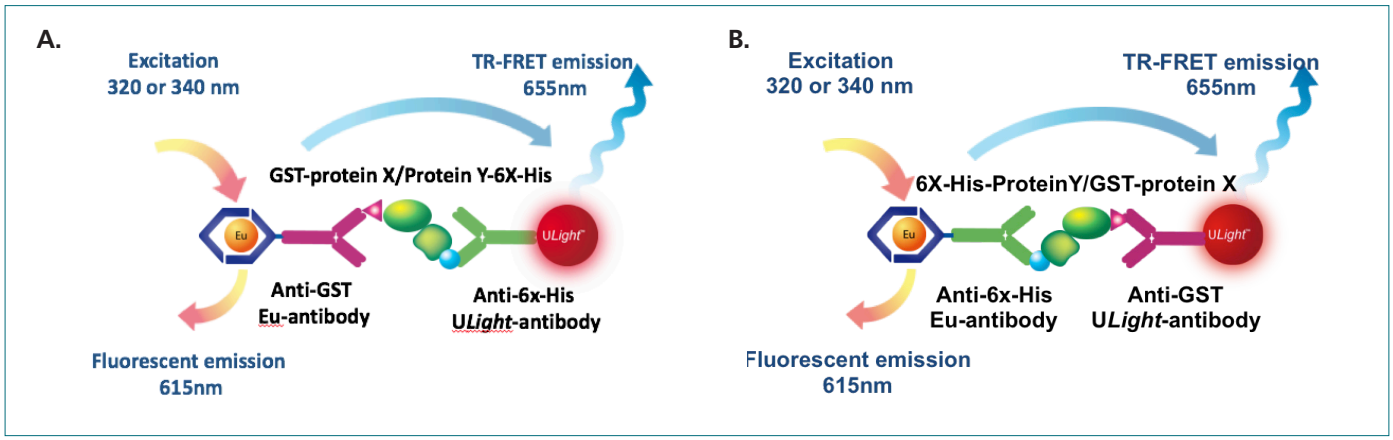

Figure 1. Illustration of a LANCE protein: protein interaction assay, using anti-GST Europium W1024 Chelate, Anti-6X-His ULight conjugate, 6X-His-Protein Y and GST-Protein (panel A). Reverse configuration is shown in panel B.

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.

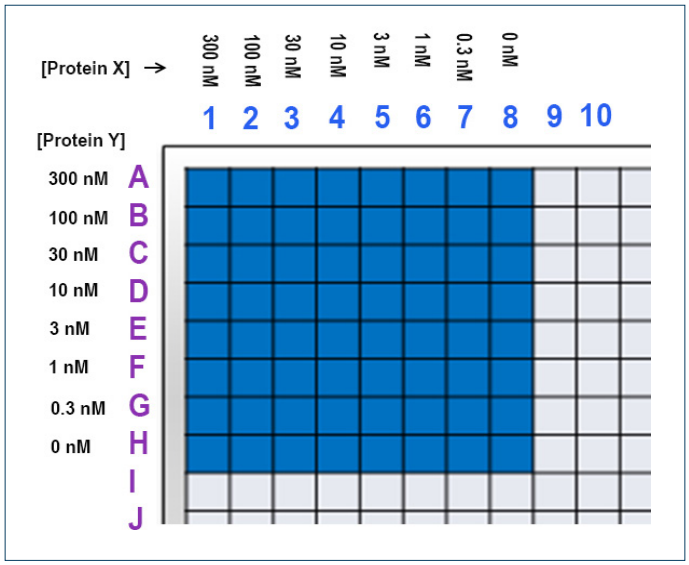

Figure 2. Plate layout utilized for one replicate of the 2D titration in Experiment #1, using 1 configuration of donor and acceptor reagents. Replicates of configuration 1 and the same concentrations of proteins using the alternate configuration 2 can be tested on the same plate.

Add 5 µL Protein X to each well
(final assay conc. 0 nM - 300 nM)

↓

Add 5 µL Protein Y to each well
(final assay conc. 0 nM - 300 nM)

↓

Incubate 60 min at desired temperature

↓

Add 5 µL Donor Reagent to each well
(final assay conc. 2 nM)

↓

Add 5 µL Acceptor Reagent to each well
(final assay conc. 100 nM)

↓

Incubate 60 min at desired temperature

↓

Read on a TR-FRET compatible reader (e.g., EnVision® Multilabel Plate Reader).
Read plate at 60, 90, 120, 180, and 240 minutes, as well as 18 and 24 hours.

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)

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

013115A_01 PKI