### Table 1. GFP² and Rluc fusion proteins

| Combination | C-terminal GFP² fusion protein | N-terminal GFP² fusion protein | C-terminal Rluc fusion protein | N-terminal Rluc fusion protein |
|-------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| 1           | Protein A                      |                                | Protein B                      |                                |
| 2           | Protein A                      |                                |                                | Protein B                      |
| 3           |                                | Protein A                      |                                | Protein B                      |
| 4           |                                |                                | Protein A                      |                                |

### Table 2. BRET² transfection matrix

| Rluc-Protein B | 0µg | 1µg | 3µg | 10µg |
|----------------|-----|-----|-----|------|
| 0µg Condition 1 | | | | |
| 1µg Condition 2 | | | | |
| 3µg Condition 7 | | | | |
| 3µg Condition 12 | | | | |
| 3µg Condition 3 | | | | |
| 3µg Condition 8 | | | | |
| 3µg Condition 13 | | | | |
| 10µg Condition 4 | | | | |
| 10µg Condition 9 | | | | |
| 10µg Condition 14 | | | | |
| 20µg Condition 5 | | | | |
| 20µg Condition 10 | | | | |
| 20µg Condition 15 | | | | |
| 40µg Condition 6 | | | | |
| 40µg Condition 11 | | | | |
| 40µg Condition 16 | | | | |

### Table 3. Cell number titration in Optiplate

| 50,000 cells | 100,000 cells | 200,000 cells |
|--------------|---------------|---------------|
| Condition 1 Non-treated | | |
| Condition 1 + Treatment | | |
| Condition 2 Non-treated | | |
| Condition 2 + Treatment | | |
| Condition 3 Non-treated | | |
| Condition 3 + Treatment | | |
Calculation #1:

\[
\text{GFP}^2 \text{ emission (515nm)} - \text{GFP}^2 \text{ emission of non-transfected cells (515nm)} \\
\text{Rluc emission (410 nm)} - \text{Rluc emission of non-transfected cells (410 nm)}
\]

= Corrected BRET$^2$ Ratio

Calculation #2:

Corrected BRET$^2$ Ratio – Correction Factor (Cf)

Where Cf =

\[
\text{GFP}^2 \text{ emission (515nm)} - \text{GFP}^2 \text{ emission of non-transfected cells (515nm)} \\
(Rluc emission (410nm) – Rluc emission of non-transfected cells (410))
\]

for the Rluc vector expressed with the empty GFP$^2$ vector