Protein-protein interaction analysis

Materials

1. 384-titer plate (Optiplate or Alphaplate) (PerkinElmer Japan, Yokohama, Japan).
2. Plate seal (AM-111) (Tokyo Garasu Kikai, Tokyo, Japan)
3. AlphaScreen Beads (Streptavidin Donor + Protein A Acceptor) (PerkinElmer Japan, Yokohama, Japan)
4. Multimode plate reader (Envision) (PerkinElmer Japan, Yokohama, Japan)

Methods

1. Prepare 15 µl of interaction mixture as follows: 1 µl of biotinylated proteins, 1 µl of AGIA (or FLAG)-tagged proteins, 1.5 µl of 10x AlphaScreen buffer (1 M Tris-HCl pH 8.0, 1% Tween20), 1.5 µl of 10 mg/ml BSA, and 10 µl of Milli-Q water. (Note 1)
2. Transfer 15 µl of the interaction mixture into 384-titer plate and seal the plate to avoid evaporation.
3. Incubate at 26℃ for 1 h.
4. Prepare 10 µl of AlphaScreen bead mixture as follows: 1 µl of 10x AlphaScreen buffer, 1 µl of 10 mg/ml BSA, 0.1 µl of acceptor beads, 0.1 µl of donor beads, 0.01 µl of anti-AGIA (or FLAG) antibody (1 mg/ml), and 7.8 µl of Milli-Q water. (Note 2,

3)

5. Add 10 µl of the AlphaScreen bead mixture to the interaction mixture in 384-titer plate and seal the plate to avoid evaporation.
6. Incubate at 26℃ for 1 h.
7. Measure AlphaScreen signal by Envision.

Note

1. You may add 50-150 mM NaCl for decreasing non-specific interaction.
2. As AlphaScreen beads are sensitive to light, operations after step 3 should be done under the weak light (lower than 100 lux).
3. Concentration of antibodies should be optimized according to their affinity and concentration.