GFP-Trap® capture surface for Biacore assays

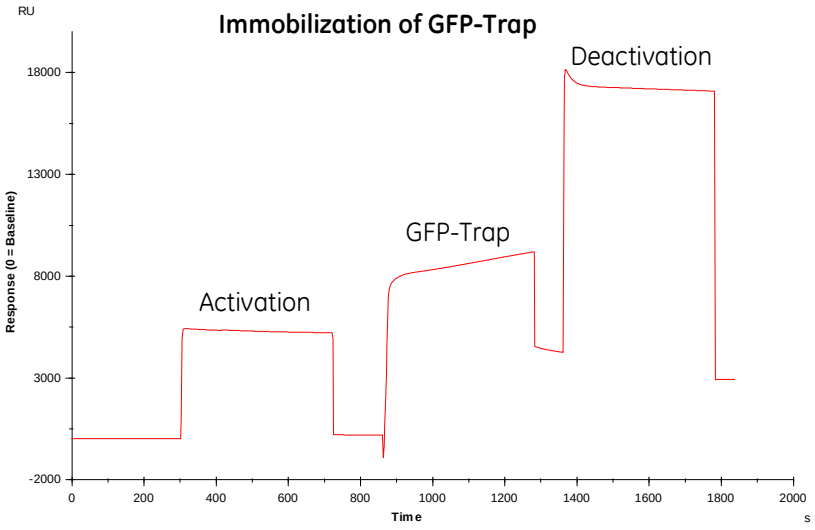
GFP-Trap is a specific and stable binding molecule for green fluorescent protein (GFP)-tagged proteins convenient for usage in a Biacore capture assay format. GFP-Trap also recognizes proteins tagged with enhanced GFP (eGFP) and yellow fluorescent protein derivatives (YFP, eYFP and Venus).

This capture system allows an easy and effective isolation and directed immobilization of fluorescent fusion proteins from complex biological samples like prokaryotic or eukaryotic cell extracts. Immobilization of these proteins enables binding studies and kinetic analysis using Biacore systems. With the GFP-Trap capture system valid data on localization, dynamics and mobility of proteins can be directly combined with interaction analysis using Biacore systems.

Protocol

This protocol describes mainly how to immobilize and regenerate GFP-Trap.

1. **Immobilization of the GFP-Trap to an immobilization level around ~3000 RU**
   - Dilute GFP-Trap to 50 µg/ml in 10 mM acetate pH 5.5
   - Coupling chemistry: Amine coupling
   - Activation time: 7 min, ligand contact time: 7 min, deactivation time: 7 min
   - Flow rate: 10 µl/min
   - Sensor Chip CM5
   - Immobilize the GFP-Trap on both the active and reference surface



2. Capture of a GFP-tagged protein

You can calculate how much ligand you need on the sensor surface to give a desired theoretical R_{max} (maximum binding capacity for analyte) using the following formula:

$$R_{max} = \frac{\text{Analyte } M_W \times R_L \times S_m}{\text{Ligand } M_W}$$

R_{max} [RU] = Maximal binding response
R_L [RU] = Immobilization level
S_m = Stoichiometric ratio
M_w [Da] = Molecular weight

For kinetic analysis use low immobilization levels, typically a theoretical R_{max} value between 50-100 RU. In practice, the experimental R_{max} is usually somewhat lower than the theoretical value.

3. Investigate binding of your analyte to the GFP-tagged protein

- Start with a pM dilution of the GFP-tagged protein.
- If the concentration of the GFP-tagged protein in the extract is not known, set up a number of 10-times dilutions in running buffer. Start testing with the lowest concentration.
- Inject the analyte over both the active (GFP-tagged protein captured to GFP-Trap) and reference surface (GFP-Trap).

4. Regenerate the GPF-tagged protein from the GFP-Trap surface

- Use Glycine pH 2.0
- Contact time: 45 s
- Flow rate: 30 µl/min

For local office contact information, visit
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