



Protocol

Determination of the extent of biotinylation- BRTA assay Assay for Positive control protein substrate kit- BIS 300

This procedure follows a typical ELISA protocol. The extent of biotinylation of the test protein is measured by comparison with known quantities of fully biotinylated MBP-AviTag protein. The standard protein and unknown proteins are adsorbed to the wells of a 96-well plate at known protein quantities. The biotin associated with the AviTag is detected by its interaction with streptavidin-conjugated alkaline phosphatase. Extraneous biotin is removed when the samples are washed after adsorption, eliminating exhaustive dialysis steps. The disadvantage of the BRTA is that some test proteins may not bind well to the plastic of the 96-well plate.

Reagents

- Fully biotinylated MBP-AviTag fusion protein, 50 μ l 1 mg/ml (**BIS-300** Avidity, L.L.C.)
- Un-biotinylated MBP-AviTag fusion protein, 50 μ l 1.95 mg/ml (**BIS-300** Avidity, L.L.C.)
- Streptavidin-Alkaline Phosphatase ([Molecular Probes](#), Eugene, OR) 2 mg/ml

Solutions

- **PBS:** 138 mM NaCl, 2.6 mM KCl in 10 mM potassium phosphate (pH 7.4)
- **PBST:** PBS plus 0.05% Tween 20
- **Blocking Solution:** PBS plus 40 μ g/ml BSA
- **Dilution Buffer:** PBS plus 0.15 μ g/ml BSA
- 40 μ g/ml Streptavidin-Alkaline Phosphatase in PBS
- **Developing Solution for alkaline phosphatase:** p-nitrophenyl phosphate in diethanolamine buffer ([Bio-Rad Laboratories](#), Hercules, CA)

Equipment

- **Corning Costar Corp** , ([Corning, NY](#)) 96-well polystyrene, high binding, flat bottom microtiter plates
- Biokinetics Reader EL312e ([Bio-Tek Instruments](#), Winooski, VT)



Procedure

1. For the standard curve, coat the wells of a 96-well polystyrene microtiter plate with 1 to 10 ng (1 ng increments) and 20 to 45 ng (5 ng increments) of fully biotinylated MBP-AviTag fusion protein using dilution buffer to bring the volume to 50 μ l.
2. Coat wells of a 96-well polystyrene microtiter plate with 1.5 to 45 ng of the protein of interest. The proportion of contaminants in the test protein samples must be determined to accurately assess the degree of biotinylation.
3. 45 ng of un-biotinylated MBP-AviTag should be added to one well as a negative control. Wells containing dilution buffer alone are used to blank the plate reader.
4. Allow the proteins to adsorb to the plate for at least 1 hr at room temperature with gentle rotational shaking.
5. Wash the plate four times with PBST.
6. Incubate plate with 300 μ l of blocking solution for 1 hr at room temperature to block non-specific binding.
7. Rinse four times with PBST.
8. Pipet 50 μ l of streptavidin-alkaline phosphatase solution into each well and incubate with gentle shaking at least 1 hr at room temperature.
9. Wash 4 times with PBST.
10. Wash the plate twice with TBS (10 mM Tris, pH 7.5, 150 mM NaCl).
11. Add 200 μ l of the developing solution.
12. Develop the plate with 200 μ l of developing solution. Monitor absorption at 405 nm every minute to assure the linearity of the alkaline phosphatase reaction. Terminate the reaction by adding 50 μ l of 2 M KOH.