
Communication

A Cost-Effective Immobilization Method for MBP Fusion Proteins on Microtiter Plates Using a Gelatinized Starch–Agarose Mixture and Its Application for Convenient Protein–Protein Interaction Analysis

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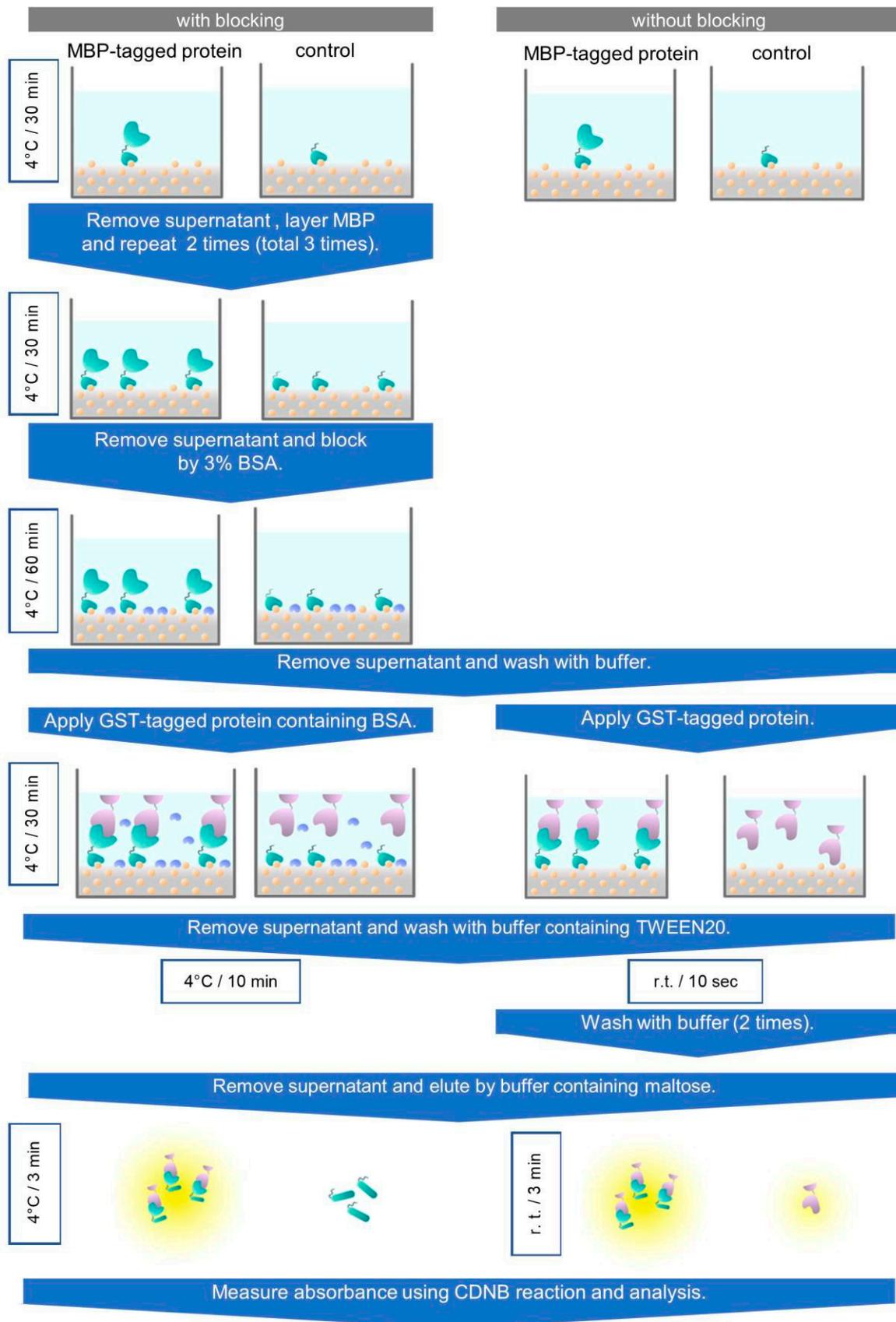
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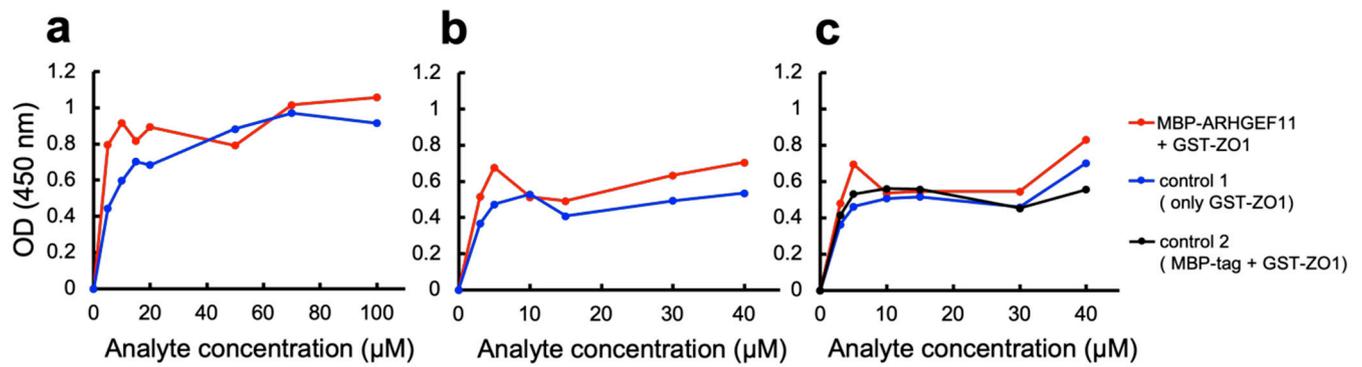
Supplementary materials

Supplementary Figure S1



Supplementary Figure S1. Comparison of a GSA-based protein–protein interaction assay using MBP- and GST-fusion proteins with and without the BSA blocking step. (left) Overview of the standard GSA-based protein–protein interaction assay with the BSA blocking step. (right) Overview of the modified GSA-based protein–protein interaction assay without the BSA blocking step. In this protocol, the GST-tagged protein is applied without BSA and then washed twice by buffer. We examined these process at room temperature, however, the difference of operating temperature seemed trivial. Without BSA-blocking, the background from control well seems to be higher than the original protocol.

Supplementary Figure S2



Supplementary Figure S2. The interaction between MBP-ARHGEF11 and GST-ZO1-ZU5 was detected by an ELISA-like method to try to estimate the K_D value. The $OD_{450\text{ nm}}$ values were plotted for each concentration of GST-ZO1-ZU5. MBP-ARHGEF11 was added as the ligand protein (red lines), while MBP without ARHGEF11 (black line) and no protein (blue lines) were as negative controls. The concentrations of GST-ZO1-ZU5 were (a) 5, 10, 15, 20, 50, and 100 μM and (b) 3, 5, 10, 15, 30, and 40 μM .