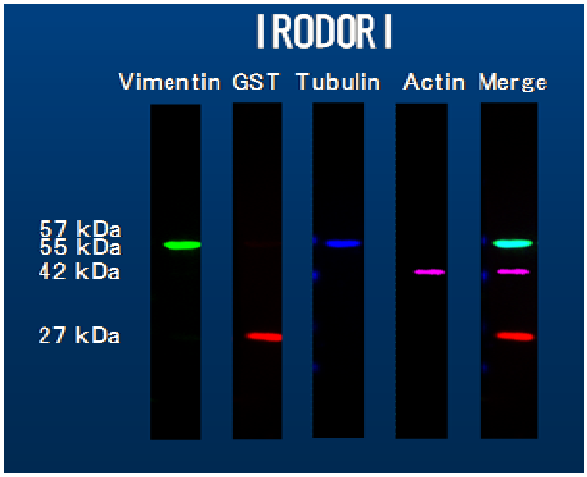
Protocol for IRODORI (Multi-color Western blot system)

Example of IRODORI



In the example 4 antigens (vimentin, GST, tubulin, actin) were detected using each antigen-specific antibodies which is attached to fluorescent MAD reagent labeled with 4 different Cy dyes.

Materials

fMAD (fluorescent MAD reagent with Cy dye-labeled):
4 different MAD types are available (Cy 2, Cy3, Cy4, Cy7).
BNC-ZZ (non labeled)
5 mM BS3 dissolved in PBS
10 mM glycine
Skim milk
TBST (0.02M Tris-HCl, 0.14M NaCl, 0.05% Tween 20, pH 7.4)
Protein A Sepharose
PBS (pH 7.4)
Antibodies

1. Preparation of antibody-fMAD complex (fMAD complex)

For multicolor western, prepare multiple complexes made of different antibody and labeled with Cy dyes of different fluorescence properties.

1) Dissolve 5µg of fMAD in adequate amount of PBS. To the fMAD solution, add 5 µL of 1mg/mL of Antibody solution*, and 5 µL of 5mM BS3 solution. Adjust the

volume to be 50 µL.

*: If the concentration of antibody solution less than 1mg/ml, increase the volume of antibody solution so that the amount of antibody should be 5 µg.

2) Incubate the solution for 30min at RT, and the at the end of incubation add 0.5µL of 10mM glycine to stop the reaction
3) Add all the solution to 100µL of Protein A Sepharose resin, incubate for15min at RT, and centrifuge at 10000 rpm for 2 min at 4℃.
4) Transfer 50µL of the supernatant add 5µg of BNC-ZZ , and incubation for 30 min at RT.
5) The antibody-fMAD complex is now ready.

2. Multi-color western

1) Prepare protein-transferred membrane by standard method.
2) Block the membrane by incubating with Blocking buffer (0.05% Skim milk dissolved in TBST) for 1hr.
3) When using multiple antibody-fMAD complexes, combine them. Then, dilute the antibody-fMAD complex solution to 500µL by Blocking buffer .
4) Incubate the membrane with the diluted antibody-fMAD complex solution for 1hr at RT.
5) Take the fluorescent (pseudo-color) image of the membrane using corresponding excitation and emission wave lengths of each antibody-fMAD complex.
6) Combine the captured pseudo-color image.

Reference

Fluorophore-labeled nanocapsules displaying IgG Fc-binding domains for the simultaneous detection of multiple antigens
M.Iijima, T. Matsuzaki, N. Yoshimoto, T. Niimi, K. Tanizawa, S.Kuroda (2011)
Biomaterials 32, 9011-9020