Using Duolink in Multiwell Plates

Protein Interaction

Recommended modifications to the Duolink In Situ Fluorescence User Manual

When using the Duolink reagents to stain cells grown in multiwell plates, a few modifications to the Duolink protocol, optimized for staining samples deposited on glass slides, have to be made in order to suit the multiwell format. In particular, the washing procedure, the preparation for imaging, and the plate scanning differ from the standard protocol. We recommend you carefully read through the Duolink In Situ Fluorescence User Manual and adjust the protocol according to the following guidelines when staining cells in 96-well multiwell plates. Additional modifications to the protocol might also be necessary to meet your specific application or assay design.

Recommended Equipment

- PerkinElmer® ViewPlate-96 Black, Optically Clear Bottom (PerkinElmer, Art no. 6005182, case of 50) or similar plates
- Plastic multiwell plate lid, adhesive plastic film or Parafilm®
- Orbital shakers for incubation and wash steps
- Cellomics ArrayScan or other high resolution laser scanning microscope
- Duolink In situ Microplate Nuclear Stain and Anti-Fade (DUO82064)
- Duolink In situ Microplate Heat Transfer Block (DUO82065)

Pre-Treatment, Blocking, and Primary Antibodies

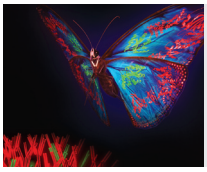
Grow cells in multiwell plates and treat (starve, stimulate etc.), wash, and fix the cells according to your standard immunostaining protocol. The conditions for your primary antibodies should be optimized with respect to sample fixation, antigen retrieval, blocking solution, antibody diluent, concentrations, and incubation temperature and time.

Incubation Steps

40 µl of reagents per well is recommended. During the incubations, cover the plate with a plastic plate lid, adhesive plastic film, or Parafilm to prevent drying of the samples. Incubation in a pre-heated Microplate Heat Transfer Block will increase staining efficiency and reproducibility between different plates and reduce edge effects. It is also recommended to place the plate on an orbital shaker (maximum 240 rpm) during the incubation steps to improve the evenness of the staining.

Wash Steps

At least 200 µl of 1x Wash Buffer A per well is recommended per wash step. Washing can be done either manually or automatic with a multiwell cell washer module. Try to remove as much remaining wash buffer before adding new reagents to avoid interference. At the same time, do not allow the samples to dry and make sure the washing is not too violent since the cells may detach. After each wash step, let the samples soak in wash buffer before aspirating, e.g. during a 2 x 5 min wash: wash with 200 µl and let soak for 5 min, aspirate, then wash in another 200 µl and let soak for 5 min, aspirate and add new reagents. During the Final Wash Step in Wash Buffer B, let the samples soak for 2 x 10 min in 1x Wash Buffer B, before proceeding to preparation for imaging.



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Preparation for imaging

For nuclear staining with DAPI and preserving PLA signals, use Microplate Nuclear Stain and Anti-Fade which contains Nuclear Staining Buffer (10x) and Anti- Fade Buffer (10x). Start this protocol after the Final Wash Step in 1x Wash Buffer B for 2 x 10 min:

a) Bring the Nuclear Staining Buffer to room temperature.

b) Add 9.0 mL of high purity water to the vial to obtain a 1x Nuclear Staining Buffer.

c) Let the samples soak in 1x Wash Buffer A for 2 min.

d) Remove the wash buffer and add 50 µl/well of 1x Nuclear Staining Buffer.

e) Incubate for 30 min at 37 °C. In the end of this incubation bring the Anti-Fade Buffer to room temperature.

f) Add 9.0 mL of high purity water to the vial to obtain a 1x Anti-Fade Buffer.

g) Let the samples soak in 1x Wash Buffer A for 2 min.

h) Remove the wash buffer and add 50 µl/well of 1x Anti-Fade Buffer.

i) Scan the plate.

To prevent evaporation during prolonged scanning, seal the plate with an adhesive plastic film. Scanned plates should be stored at 4 °C, protected from light. The signals will remain for a week if stored at 4 °C.

Plate Scanning

Use scanning settings appropriate for your microscope and make sure the correct filters are selected for image acquisition. At least 20x magnification should be used. A fixed exposure time in the signal channel is required to be able to compare data between wells and scanning should preferably be done at a resolution of 0.25 µm/pixel but at least 0.45 µm/pixel.

Recommendations for 384-Well Plates

Use PerkinElmer® ViewPlate-384 Black, Optically Clear Bottom (PerkinElmer, Art no. 6007460, case of 40) or similar plates and at least 15 µl of reagents per well. Be careful with washings since bubbles easily form, using TBS without TWEEN® 20 may help. Bubbles can also be removed by centrifuging the plate briefly. Uneven staining across the plate can be avoided by putting the plate on ice during the pipetting steps and then put the plate in a pre-heated Microplate Heat Transfer Block during the 37 °C incubations.

Reference

Leuchowius KJ, Jarvius M, Wickström M, Rickardson L, Landegren U, Larsson R, Söderberg O, Fryknäs M & Jarvius J. High content screening for inhibitors of protein interactions and post-translational modifications in primary cells by proximity ligation. Mol Cell Proteomics. 9,178–183 (2010).