Duolink® In Situ

GUIDELINES

Using Duolink with Shandon Sequenza immunostaining system

RECOMMENDED MODIFICATIONS TO THE DUOLINK IN SITU FLUORESCENCE USER MANUAL (DOC NO. 0650) OR DUOLINK IN SITU BRIGHTFIELD USER MANUAL (DOC NO. 0656)

When using the Duolink reagents to stain samples with the Shandon Sequenza® immunostaining system a few modifications to the Duolink protocol, optimized for manual staining of samples deposited on glass slides, have to be made in order to suit this system. In particular, the primary antibody concentrations and reagent and washing volume differ from the standard protocol. We recommend you to carefully read through the User Manual (Doc no. 0650 or 0656) and adjust the protocol according to the following guidelines when combining Duolink with the Shandon Sequenza immunostaining system. Additional modifications to the protocol might also be necessary to meet your specific application or assay design.

RECOMMENDED EQUIPMENT

- Shandon Sequenza slide rack (Fisher Scientific, Art no. 73310017)
- Shandon Sequenza disposable coverplates (Fisher Scientific, Art no. 72110017, case of 25, Art no. 7219950, case of 50, Art no. 72110013, case of 250)
- Microscope slides with fixed cells or tissue

Note: Do not use slides where the sample is delimited by a well or hydrophobic barrier.

PRE-TREATMENT, BLOCKING AND PRIMARY ANTIBODIES

The conditions for your primary antibodies should be optimized with respect to sample fixation, antigen retrieval, blocking solution, antibody diluent and incubation temperature and time. Note: You will need a higher concentration of the primary antibodies with the Shandon Sequenza system compared to when using Duolink for manual staining to receive a comparable number of signals. All steps, starting from blocking to the Final Wash Step, should be performed when the slides are mounted to the Shandon Sequenza coverplates and placed in the slide rack. When mounting the slide to the coverplate, add 500 µl of PBS or sample buffer to the coverplate to create a liquid surface between the slide and the coverplate.

INCUBATION STEPS

100 µl of reagents per slide is recommended. All Duolink reagent stocks should be diluted to the concentrations described in the User Manual. During the 37°C incubations put the lid on top to prevent drying of the samples.

WASH STEPS

Follow the washing recommendations in the User Manual but add 1000 µl of wash buffer/slide per wash step, e.g. during a 2x5 min wash, add 1000 µl of wash buffer/slide, wait for 5 min, then add another 1000 µl of wash buffer/slide and wait for 5 min before you add new reagents.

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