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Product Information

Duolink® PLA Control Kit - PPI

Catalog Number **DUO92202**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Duolink® PLA Control Kits are intended for use with Duolink PLA reagents to ensure proper function of reagents and Duolink PLA staining technique.

Components

Each kit contains two Millicell EZ 8 well chamber slides (Catalog Number PEZGS0816) with pre-fixed SK-OV3 (Catalog Number 91091004) cells, plus sufficient primary antibody for positive (both primary antibodies) and negative (each primary antibody separately and no primary antibody) technical controls.

Duolink PLA control slides 2 slides
(Catalog Number DUO82202)

Mouse anti-EGFR antibody 25 µL
(Catalog Number E3138)

Rabbit anti-ErbB2/HER2 antibody 10 µL
(Catalog Number HPA001383)

Reagents and Equipment Required but Not Provided.

1× Phosphate buffered saline (PBS, Catalog Number P3813)

Triton™ X-100 (Catalog Number X100)

PAP pen for immunostaining (Hydrophobic pen, Catalog Number Z377821)

Duolink PLA Blocking Buffer (Catalog Number DUO82007)

For optimal results, use this kit with Duolink PLA Probe Anti-Rabbit PLUS (DUO92002), Duolink PLA Probe Anti-Mouse MINUS (DUO92004), and a Duolink PLA Detection Kit of the user's choosing or a Duolink PLA Starter Kit Mouse/Rabbit (DUO92101 or DUO92102).

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

PBS wash buffer – Dissolve the contents of one pouch of 1× PBS (Catalog Number P3813) in high purity water and QS to 1 L.

Permeabilization solution – Prepare 0.2% Triton X-100 in 1× PBS by adding 0.2 mL of Triton X-100 (Catalog Number T8787) per 100 mL of 1× PBS.

Primary antibody dilutions – Both primary antibodies are supplied as stock solutions. Dilute the mouse anti-EGFR antibody and the rabbit anti- ErbB2/ HER2 antibody in Duolink PLA antibody diluent (Catalog Number DUO82008) to final concentrations of 1:100 and 1:5000, respectively, immediately before use (Procedure, step 8).

a. Pre-dilute the anti-ErbB2/HER2 antibody 1:50 in antibody diluent.
b. Further dilute both primary antibodies 1:100 in antibody diluent as shown in the Table 1.
c. Use 40 µL per well in duplicate wells as indicated in the table.

Table 1.
Antibody Dilutions

Component	Tube 1	Tube 2	Tube 3	Tube 4
Mouse Anti-EGFR (E3138)	1 µL	1 µL	–	–
Rabbit Anti-HER2* (HPA001383)	1 µL	–	1 µL	–
Antibody Diluent	98 µL	99 µL	99 µL	100 µL
Well Placement	1 and 5	2 and 6	3 and 7	4 and 8

*Use 1:50 prediluted stock of anti-HER2 antibody from step a.

Storage/Stability

Store the slides and primary antibodies at –20 °C. **Do not store diluted primary antibodies**

Procedure
The Duolink PLA Fluorescence Protocol can be found at sigma.com/duolink-fluorescense.

SK-OV3 cells have already been plated on 8 well glass chambered slides, stimulated with EGF to induce EGFR-HER2 interaction, fixed, and frozen. Before starting the Duolink PLA protocol, the cells must be permeabilized to allow access of the primary antibodies into the cells. Start this procedure prior to the Blocking step on page 2 of the Duolink PLA Fluorescence Protocol.

Note: To minimize cross-contamination of solutions from well to well, use a hydrophobic pen (Catalog Number Z377821) or aspirate between the wells prior to addition of reagents.

1. Wash slides of pre-fixed cells (Catalog Number DUO82202) with PBS wash buffer for 2 minutes at room temperature in a Coplin jar.
2. Tap off excess PBS wash buffer and permeabilize using 40–50 mL of permeabilization solution in a Coplin jar.
3. Incubate for 10 minutes at room temperature.
4. Wash slides twice with PBS wash buffer for 2 minutes at room temperature in a Coplin jar, shaking gently.
5. Tap off excess PBS wash buffer, aspirate between wells, and add 1–2 drops (or 40 µL) of Duolink PLA Blocking Buffer (Catalog Number DUO82007) per well.
6. Incubate for at least 1 hour at 37 °C in a humidity chamber.
7. Dilute the primary antibodies, see Table 1.
8. Tap off excess Blocking Buffer, aspirate between wells, and add 40 µL of the appropriate primary antibody solution per well in duplicate wells as indicated in Table 1.
9. Incubate for 2 hour at 37 °C or overnight at 4 °C in a humidity chamber.
10. Proceed with the Duolink PLA Fluorescence Protocol, starting at the Duolink PLA Probe Incubation step.

Note: Due to differential expression of EGFR and HER2 by SK-OV3 cells, Duolink PLA signal levels may vary from cell-to-cell. In addition, few PLA signals may be detected in single primary antibody controls. However, a significant difference between the positive (wells 1 and 5) and negative (remaining wells) controls should be readily apparent.

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