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Duolink® PLA Probemaker Guide

This guide describes the use of the Duolink® *In Situ* Probemaker for the conjugation of PLA oligonucleotides (PLUS or MINUS) to any antibody for use in Duolink® PLA experiments. Probemaker-generated PLA antibodies can be used in combination with or independent from standard Duolink® PLA Probes, as long as a PLUS PLA probe and a MINUS PLA probe are used.

Applications

Duolink® Probemaker is useful for situations when the use of current Duolink® PLA Probes alone is not feasible or ideal. This section provides common applications of Duolink® Probemaker.

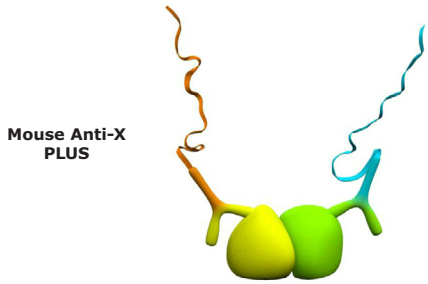
1. Use of Primary Antibodies from the Same Species

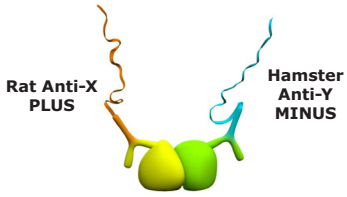
When using two primary antibodies derived from the same species, Duolink® Probemaker can be used to conjugate one of the antibodies with the PLA PLUS oligonucleotides and the other with the PLA MINUS oligonucleotides. When using only directly conjugated primary antibodies, no secondary antibody or Duolink® PLA Probe is needed and this step in the PLA protocol should be eliminated.

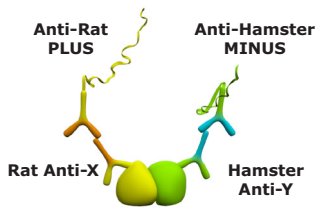
2. Use of Primary Antibodies from any Species

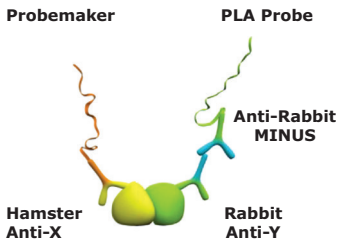
Current offerings of Duolink® PLA Probes are PLA-conjugated secondary antibodies that recognize IgG from mouse, rabbit, or goat. When using two primary antibodies derived from species other than mouse, rabbit, or goat, Duolink® Probemaker can be used to label either the primary antibodies or the appropriate secondary antibodies with PLA oligonucleotides. Conjugate one of the antibodies with the PLA PLUS oligonucleotides and the other antibody with the PLA MINUS oligonucleotides.

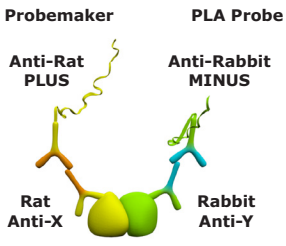
When only one primary antibody is derived from a species other than mouse, rabbit, or goat, a combination of Duolink® Probemaker-generated PLA antibodies and standard Duolink® PLA Probes can be used, as long as a PLUS PLA probe and a MINUS PLA probe are used.





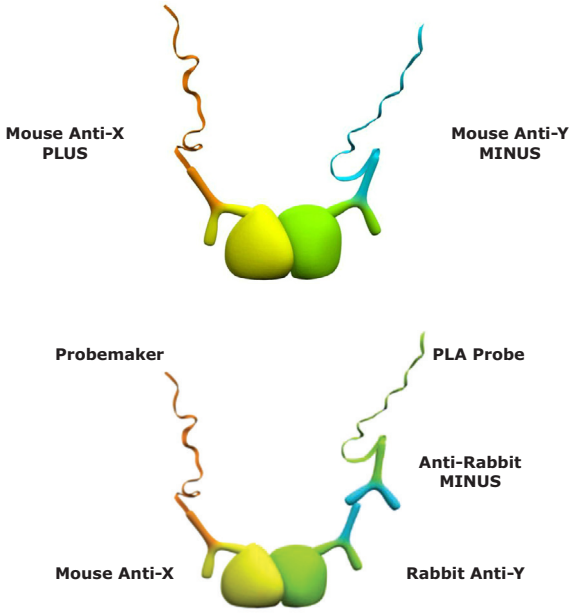






3. **Use of Primary Antibody Derived from the Same Species as Tissue Sample (e.g., "Mouse on Mouse")**

Staining of mouse tissue using a mouse-derived primary antibody often results in high background caused by the secondary antibody binding to endogenous mouse IgG in the tissue being stained, and to Fc receptors on B cells, plasma cells and macrophages. To bypass the use of anti-mouse secondary antibodies and reduce background, Duolink® Probemaker can be used to directly conjugate the mouse-derived primary antibody with PLA oligonucleotides.



Materials and Equipment

Duolink® Probemaker Reagents

Duolink® Probemaker allows you to create custom PLA oligo-conjugated antibodies, which can be used in combination with or independent from standard Duolink® PLA Probes, as long as a PLUS PLA probe and a MINUS PLA probe are used. Each Duolink® PLA Probemaker kit contains reagents to conjugate 20 µg of antibody at a concentration of 1 mg/mL. The reagents include the following:

a. **Duolink® PLA oligonucleotides** – One vial with lyophilized, activated oligonucleotides (PLUS or MINUS)

b. **Conjugation Buffer** – For buffering the conjugation reaction

c. **Stop Reagent** – For stopping the conjugation reaction

d. **Storage Solution** – For preserving the conjugated antibody (PLA probe)

e. **20x Assay Reagent** – To be added to experimenter-optimized antibody diluent if necessary

f. **Blocking Solution** – For blocking sample prior to primary antibody incubation

g. **PLA Probe Diluent** – For diluting conjugated antibody (PLA probe) to the final assay concentration

NOTE: Store all reagents at -20°C. Once the antibody is conjugated, store at 4°C.

Additional Materials

1. **Primary or secondary antibody** – For conjugation with PLA oligonucleotides (PLUS and/or MINUS). To participate in conjugation, the antibodies must fulfill the following criteria:

   a) The antibody to be conjugated must have a concentration of 1 mg/mL. 20 µg (=20 µL) of antibody is needed per conjugation.
   NOTE: If a monoclonal antibody is used, it should be Protein A or Protein G affinity purified.

   b) The antibody must be in an amine-free buffer, ideally PBS. The buffer should be carrier- and preservative-free, but may contain up to 0.1% BSA, 5% trehalose, and 0.02% sodium azide.
   NOTE: If the composition of the buffer that the antibodies are stored in is unknown, it is recommended that dialysis or buffer exchange takes place prior to conjugation.

NOTE: Concentrating very diluted antibodies prior to Duolink® In Situ Probemaker conjugation is not recommended unless you have large amounts since losses are very high with filter type concentrators.

2. 1x PBS – For buffer exchange of antibody, if needed. The recommended procedure for buffer exchange is as follows:

NOTE: This procedure is not for reducing high concentrations of BSA or other macromolecules.

   1) Pre-equilibrate a spin column with 1x PBS by first spinning the column at 3000 rpm for 1 min, then add 400 µL of 1x PBS and spin again for 1 min and repeat 4 times. Place the column in a new microfuge tube.

   2) Add your antibody (12–50 µL) to the column and spin again for 2 min at 3000 rpm. The concentration of the collected antibody should be verified by OD. 1 mg/mL should have an OD 280 nm of 1.4.

NOTE: Microcon-10 Centrifugal Filter Units work well (MilliporeSigma, MRCPRT010).

Duolink® PLA Probemaker Conjugation Protocol

The Conjugation Protocol describes how to conjugate PLA oligonucleotides (PLUS or MINUS) to any antibody. Before starting, ensure that the antibody to be conjugated is in an amine-free buffer at a concentration of 1 mg/mL. The antibody buffer should be carrier- and preservative- free, but may contain up to 0.1% BSA, 5% trehalose, and 0.02% sodium azide. Vortex all liquid reagents before use.

1. Add 2 µL of Conjugation Buffer to 20 µL of the antibody to be conjugated. The antibody concentration should be 1 mg/mL.
2. Mix by gently pipetting.
3. Transfer the antibody solution to one vial of lyophilized oligonucleotides (PLUS or MINUS). **NOTE:** Add the antibody solution immediately after opening the vial of lyophilized oligonucleotides.
4. Mix by gently pipetting.
5. Incubate at room temperature overnight.
6. Add 2 µL of Stop Reagent to the reaction.
7. Incubate at room temperature for 30 minutes.
8. Add 24 µL of Storage Solution and if necessary, other reagents to stabilize the specific antibody.
9. After stabilization is complete, the PLA probe can be stored in the Storage Solution at 4°C.

Duolink® PLA Detection Protocol

The Duolink® PLA Fluorescence Protocol or the Duolink® PLA Brightfield Protocol can be used with Probemaker-generated antibodies in combination with or independent from standard Duolink® PLA Probes, as long as a PLUS PLA-conjugated antibody and a MINUS PLA-conjugated antibody are used. Please note the following changes that may affect your experiment based on your PLA probes:

1. The **PLA Probe Diluent** found in the Probemaker kit should be used in substitution of the **Duolink® Antibody Diluent** in both the Fluorescence and Brightfield protocols.
2. OPTIONAL: When using a custom antibody diluent instead of the PLA Probe Diluent provided, it is recommended that **20x Assay Reagent** be diluted 1:20 in your custom antibody diluent prior to use with PLA-conjugated antibodies.
3. Incubate the PLA-conjugated antibodies at the appropriate steps in the Fluorescence and Brightfield protocols. If **primary antibodies** were used for conjugation, use them during the Primary Antibody Incubation step (Fluorescence, step 2; Brightfield, step 3). If **secondary antibodies** were used for conjugation, use them during the PLA Probe Incubation step (Fluorescence, step 3; Brightfield, step 4).
4. When using only **directly conjugated primary antibodies**, no secondary antibody or Duolink® PLA Probe is needed. Thus, skip the PLA Probe Incubation step in the Fluorescence (step 3) and Brightfield (step 4) protocols. For any other combination of PLA probes, the protocols can be followed as written.

References

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2. Söderberg O, Gullberg M, Jarvius M , Ridderstråle K, Leuchowius KJ, Jarvius J, Wester K, Hydbring P, Bahram F, Larsson LG, and Landegren U. Direct observation of individual endogenous protein complexes PLA by proximity ligation. Nat Methods, 3, 995-1000 (2006).
3. Gullberg M, Gustafsdottir SM, Schallmeiner E, Jarvius J, Bjarnegård M, Betsholtz C, Landegren U, and Fredriksson S. Cytokine detection by antibody-based proximity ligation. Proc Natl Acad Sci USA, 101, 8420-24 (2004).
4. Fredriksson S, Gullberg M, Jarvius J, Olsson C, Pietras K, Gustafsdottir SM, Östman A, and Landegren U. Protein detection using proximity-dependent DNA ligation assays. Nat Biotechnol, 20, 473-77 (2002).

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