
SOP-P044**Labeling Antibodies for Cervical Cancer Cells**

Objective: To label antibodies using Kits

Label the MTM Labs p16^{INK4a} antibody with the Lightning-Link R-PE Kit (Cat#703-0010)

Label the MTM Labs MCM5 antibody with the Lightning-Link Cy5 Kit (Cat#781-0010)

Procedure: Before adding any liquids to the glass vials, tap the vials first to knock down any chunks of lyophilized material that may be up on the sides.

P16^{INK4a} Antibody:

The LL-PE kit recommends labeling 50-60ug of antibody in a volume of 40ul. The concentration of the MTM labs p16^{INK4a} antibody is 4.15ug/ul.

Combine **14.46ul** of the antibody with **25.54ul** of PBS which makes a total of 40uls in to an eppendorf tube. (Use Clean Dulbecco's PBS from Sigma Cat#D8537, 500ml)

LL-Modifier reagent requires 1ul of modifier for every 10ul of antibody to be labeled.

So, p16^{INK4a} is in a 40ul volume, add **4ul** of the LL-Modifier. Add to the eppendorf tube containing the antibody and PBS. (Make sure the LL-Modifier reagent is thoroughly mixed with the antibody by pipetting up and down, but try not to introduce bubbles.)

Add this volume to the PE glass vial containing the dye. Be sure to mix well without causing bubbles.

Label properly with antibody and date. eg: p16 / 120821

Let stand for at least 3 hours (or overnight) protected from the light and at room temperature.

After the 3 hours or the next day, add 1ul of the LL-Quencher reagent for every 10ul of antibody that was in original volume to be labeled. So for the p16^{INK4a} /LL_PE, add **4uls**.

Let stand for at least 30 minutes minimum before using the labeled antibody. If not to be used right away, store labeled antibodies in their glass vials in 4C. Be sure to label the glass vial with the stain and date.

MCM5 Antibody:

The LL-Cy5 kit recommends labeling 100-200ug of antibody in a 40-100ul volume. The concentration of the MCM5 antibody that we are currently using is at 100ug/ul, so the easiest thing to do is just use the whole tube of antibody.

The MCM5 is in a volume of 100ul, so add 10ul of the LL-Modifier directly to the commercial antibody tube. (Make sure the LL-Modifier reagent is thoroughly mixed with the antibody by pipetting up and down, but try not to introduce bubbles.)

Add this volume to the Cy5 glass vial containing the dye. Be sure to mix well without causing bubbles.

Label properly with antibody and date. eg: MCM5 / 120821

Let stand for at least 3 hours (or overnight) protected from the light and at room temperature.

After the 3 hours or the next day, add 1ul of the LL-Quencher reagent for every 10ul of antibody that was in original volume to be labeled. So for the MCM5/LL-Cy5, add **10uls**.

Let stand for at least 30 minutes minimum before using the labeled antibody. If not to be used right away, store labeled antibodies in their glass vials in 4C. Be sure to label the glass vial with the stain and date.

Created by: Cheryl Holdman

Verified by: _____ **Date:** 8/21/2012

Print Name

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