SpeedAb Western Blot Kit

General Notes

This protocol outlines only the labeling of primary antibodies and the detection of target proteins on the membrane. SDS-PAGE and membrane transfer should be performed according to your standard procedures. A membrane blocking step is not required.

This Kit contains two components that speed up the Western blot workflow significantly by reducing the number of steps and antibody incubation time.

1. **FlexAble HRP Antibody Conjugation Kit**
   (for Rabbit IgG / for Mouse IgG1, IgG2a, or IgG2b)

   FlexAble HRP Antibody Labeling Kits are a fast and easy solution to label primary antibodies with HRP to facilitate 1-step detection without a secondary antibody.

   The FlexLinker included in the Kits is specific to IgG species and subtype. Please make sure you use the correct Kit with your rabbit or mouse primary.

2. **Blotting Accelerator**

   This ready-to-use solution is specifically used for membrane blocking and antibody dilution in Western blot experiments. Containing special organic compounds, the Blotting Accelerator is designed to significantly shorten the antibody incubation times to around 25 minutes or less per antibody, which will improve experimental efficiency, especially when combined with FlexAble HRP Kits.

Please refer to the product pages on our website if you want to learn more about each component of this kit.

Fast WB Protocol

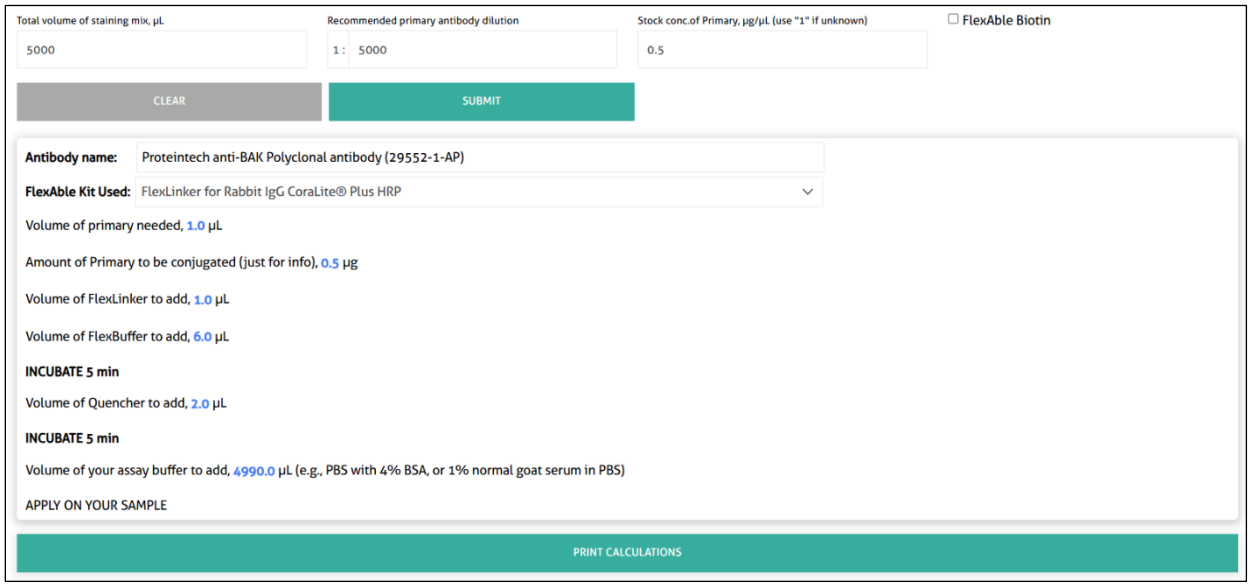
Note: To save time, we recommend labeling your primary antibody with the included FlexAble HRP kits during the SDS-PAGE or transfer step. If you need assistance with calculating respective volumes, we recommend using our FlexAble calculator tool. https://www.ptglab.com/FlexCalc

An example is shown on the following page.

To not waste antibody, we recommend to label only the amount of primary antibody used in the subsequent WB experiment.

Exemplary calculation for a primary antibody

For a final recommended dilution of 1:5,000 and an antibody stock concentration of 0.5 µg/µl in a final volume of 5 ml Blotting Accelerator (sufficient for most membrane incubations; this volume can be scaled up or down).



Note: 1 FlexAble reaction (1rxn) will label 0.5 µg of antibody using 1 µl FlexLinker. For recommended dilutions >1:5,000, we suggest to reduce the volume of primary not any further and use 1 µl of primary antibody + 1 µl of FlexLinker. For recommended dilutions <1:5,000, volumes should be scaled up accordingly.

Before you begin labeling:

- Equilibrate all reagents to room temperature (~22°C).
- Make sure to gently mix primary antibody with FlexLinker, FlexBuffer, and FlexQuencher during the labeling process by pipetting up and down or gently flicking it with your fingers. Do not vortex.

Labeling of primary antibody:

1. Calculate amounts of antibody and FlexLinker (HRP) needed for your experiment.
2. Mix the calculated volume of your primary antibody with the calculated volume of FlexLinker in a 1.5 ml Eppendorf tube and add FlexBuffer if applicable.
3. Incubate for 5 min at room temperature.
4. Add the calculated volume of FlexQuencher to quench unbound FlexLinker.
5. Incubate for 5 min at room temperature.

The antibody is now labeled and can be further used for detection.

Note: The volume of primary antibody used should follow the manufacturer's recommended dilution for Western blotting. In general, the same dilution as for conventional Western blot analysis can be applied with this kit.
When working with a new antibody, it is advisable to determine the optimal dilution via a dilution series.

Detection of protein of interest on membrane:

6. Mix the HRP labeled primary antibody with equilibrated Blotting Accelerator to achieve your desired / recommended antibody dilution. For that, please account for the dilution already done in the previous steps (in this example, the antibody was already diluted 1:10 and would require a 1:500 dilution).
   (5 ml Blotting Accelerator is sufficient for membrane incubation in most cases; this volume is scalable.)
7. Incubate the membrane in the Blotting Accelerator + antibody mixture for a minimum of 15 min at room temperature while slowly shaking.
8. Briefly rinse the membrane with PBS-T or TBS-T and wash three times for 1-2 min while shaking.
9. For detection, incubate with chemiluminescent HRP substrate at desired sensitivity.

Proteintech ECL Substrates	**Cat#**
SignalBright Max Chemiluminescent Substrate	PK10013
SignalBright Pro Chemiluminescent Substrate	PK10011

Please note:

- If you use this product for the first time, you may need to titrate the antibody dilution ratio.
- The blotting accelerator contains the preservative ProClin 300. Please wear a lab coat and disposable gloves.
- This product is for laboratory scientific research use only.