OHAUS®

Preparation and Execution of a Western Blot Experiment

Developed by OHAUS Corporation

Preparation and Execution of a Western Blot

Accurate sample preparation can improve the outcome of Western Blot procedures. Western blots are one of the most common laboratory procedures and are used to determine both protein size and expression levels. In addition to other sample manipulation, Western blots rely primarily on both a denaturing of the proteins as well as gel electrophoresis. Opened protein structures will move through an electrically charged, porous gel, over a short period of time. Larger proteins will generally not move as far as smaller structures, hence isolating different proteins based on size.

Preparing Adherent Cells

1. When confluent, wash the cell culture with phosphate buffered saline (PBS). Gently swirl then Aspirate the PBS.
2. Add fresh PBS and use a cell scraper to dislodge the cells. Pipette the mixture into microcentrifuge tubes.
3. Centrifuge at 1500 RPM for 5 minutes using the OHAUS Frontier 5515R Series Micro Centrifuge and discard the supernatant.
4. Add 180µL of ice-cold cell lysis buffer with 20µL fresh protease inhibitor cocktail.
5. Incubate for 30 minutes on ice, and then clarify the lysate through centrifugation for 10 minutes at 12,000 RPM, at 4 °C.
6. Transfer the supernatant to a fresh tube and store on ice or frozen at -20 °C or -80 °C.
7. Measure the concentration of protein using a spectrophotometer.

Sample Preparation

1. Using the formula concentration = mass / volume, determine the volume of protein extract to ensure 50µg is to be added to each well of the electrophorese gel.
2. Attach the base plate accessory and the 12mm test tube uni-block to the OHAUS Guardian 5000 Hotplate Stirrer. Turn on the device and set it at 100 °C.
3. Add 5µL sample buffer to each sample. Ensure that each sample is mixed well.
4. Heat the samples when the hotplate reaches 100 °C for 5 minutes.

Gel Preparation

1. After preparing the 10% stacking gel solution, assemble the rack for gel solidification.
2. Add stacking gel solution carefully until the appropriate level is reached. Wait for 15–30 minutes until the gel turning solidified.
3. Overlay the stacking gel with the separating gel.
4. Insert the comb, ensuring that there are no air bubbles. Wait approximately 20 minutes for the gel to solidify.

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Electrotransfer

1. Cut 6 filter sheets to fit the measurement of the gel, and one polyvinylidene fluoride (PDVF) membrane with the same dimensions.
2. Wet the sponge and filter paper in transfer buffer, and wet the PDVF membrane in methanol.
3. Separate glass plates and retrieve the gel.
4. Create a transfer sandwich.
5. Relocate the sandwich to the transfer apparatus, which should be placed on ice to maintain 4 °C. Add transfer buffer to the apparatus, and ensure that the sandwich is covered with the buffer. Place electrodes on top of the sandwich, ensuring that the PVDF membrane is between the gel and a positive electrode.
6. Transfer for 90 minutes.

Blocking and Antibody Incubation

1. Block the membrane with 5% skim milk in TBST* for 1 hour.
2. Add primary antibody in 5% bovine serum albumin and incubate overnight in 4 °C on an OHAUS Extreme Environment Shaker.
3. Wash the membrane with TBST for 5 minutes. Do this 3 times.
4. Add secondary antibody in 5% skim milk in TBST, and incubate for 1 hour.
5. Wash the membrane with TBST for 5 minutes. Do this 3 times
6. Prepare ECL mix. Incubate the membrane for 1–2 minutes.
7. Visualize the result in the dark room through use of a UV spectrum lighting source.

OHAUS Products Used Within This Procedure

OHAUS Frontier 5515R Series Micro Centrifuge

OHAUS Guardian 5000 Hotplate Stirrer

OHAUS Extreme Environment Shaker