western blot

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Abstract

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Protocol

Step 1.

Preparation of lysate from endometrial tissue，us T-PER Tissue Protein Extraction Reagent，Remove a small volume of lysate to perform a protein quantification assay. Determine the protein concentration for each tissue lysate，us BCA Protein Assay Kit.

Step 2.

Load equal amounts of protein into the wells of the SDS -PAGE gel, along with molecular weight marker， 60 ug total proteins per pore，Run the gel for 90min at 100 V，，Gel percentage ，separation gel 10%，spacer gel 5%.

Step 3.

Activate PVDF with methanol for 1 min and rinse with transfer buffer before preparing the stack，Run at 100 V for 90min.

Step 4.

Wash the membrane in three washes of TBST，5 percent skimmed milk powder，5 min each.

Step 5.

Block the membrane for overnight at 4°C using blocking buffer. Wash the membrane in four ashes of TBST, 5 min each.

Step 6.

Incubate the membrane with the recommended dilution of conjugated secondary antibody in blocking buffer at room temperature for 1 h. Wash the membrane in five ashes of TBST, 5 min each，

Step 7.

Acquire image using normal image scanning methods for colorimetric detection.

Step 8.

Step 9.