General Protocols

Immunoblotting Protocol:

After transferring to nitrocellulose membrane:

1. Block non-specific binding: incubate in PBS, containing 1% BSA at + 4°C, shaking gently for 30-60 minutes.
2. Incubate with primary antibodies: 5µg/ml of primary antibody solution in PBST for 1 hour at +37°C, shaking gently.
3. Washing procedure: wash in PBST for one minute, three times at room temperature (RT).
4. Incubate with anti-mouse IgG HRP-conjugate for one hour at +37°C in PBS-A0,2% T.
5. Washing procedure: wash in PBST for one minute, three times at RT.
6. Enzyme reaction: in 10 ml PBS dilute 2.5 mg 3,3'-diaminobenzidine, 7.5 mg 4-chloro-1-naphthol and 3µl 30% H_2O_2. Incubate membrane for 5-15 minutes at RT. Wash with water and dry.

ELISA Protocol:

1. Primary antibodies are coated on the 96-well EIA plate at 5µg/ml concentration in phosphate buffered saline (PBS, pH 7.4) overnight at + 4°C.
2. Washing procedure: wash three times, 200µl PBST per well for one minute at RT.
3. Two-fold serial dilution of the antigen is applied by 100µl per well and incubated for 1 hour at +37°C.
4. Washing procedure: wash three times, 200µl PBST per well for one minute at RT.
5. Incubation with HRP-conjugated secondary antibodies: 100µl per well in PBST for 1 hour and +37°C. Secondary antibody dilution corresponds to 0.2-1µg/ml in terms of antibody concentration.
6. Washing procedure: wash five times, 200µl PBST per well for one minute at RT.
7. Enzyme reaction: TMB Liquid Substrate for ELISA (Sigma, T0440) is applied by 100µl per well and incubated for 15 minutes at RT.
8. The reaction is stopped by sulfuric acid and the absorbance is read at 450 nm.

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