Protocols

Welcome to the Protocols section of our website. Here, we provide detailed, step-by-step protocols to assist researchers working with biological models, biochips, antibodies, and gene and cell therapies. Please refer to the specific sections below for comprehensive instructions related to your research needs.

1. Biological Models

(1) Cellular Models

Protocol for Culturing Immortalized Cell Lines:

- Thaw cells quickly in a 37°C water bath.
- Transfer the cells to 10 mL of pre-warmed complete growth medium.
- Centrifuge at 300g for 5 minutes, discard the supernatant, and resuspend the cell pellet.
- Plate cells in an appropriate cell culture flask and incubate at 37°C with 5% CO_2.
- Monitor cell confluency daily, and split cells when they reach 80-90% confluency.

(2) Mouse Models

Breeding Protocol for Knockout Mice:

- Set up breeding pairs of heterozygous mice (Knockout +/-).
- Monitor females daily for signs of pregnancy and record the breeding date.
- After birth, genotype pups using tail snips or ear punches.
- PCR is used to confirm the presence of wild-type, heterozygous, or knockout alleles.

(3) Organoid Models

Organoid Culture Protocol (Pancreatic Organoids):

- Prepare Matrigel-coated plates for 3D culture.
- Isolate pancreatic stem cells from donor tissue and resuspend in organoid culture medium.
- Seed cells into Matrigel domes and incubate at 37°C.
- Replace culture medium every 2-3 days.
- Passage organoids when they reach sufficient size by mechanical dissociation.

2. Biochips

(1) Solid Biochips

Protein Immobilization Protocol on Solid Biochips:

- Clean the biochip surface with ethanol or plasma cleaning.

- Activate the surface with amine-reactive chemistry (e.g., NHS/EDC).
- Dilute protein sample in binding buffer and apply to the activated biochip surface.
- Incubate at room temperature for 1-2 hours.
- Wash the biochip with PBS to remove unbound protein, then block remaining reactive groups.

(2) Liquid Biochips

Flow Assay Protocol for Liquid Biochips:

- Prepare sample and reagents, ensuring all components are in the appropriate concentration.
- Load the liquid biochip device with the sample and run it through the microfluidic system.
- Use the appropriate detection system (fluorescence or chemiluminescence) to capture data.
- Analyze the results using compatible software for quantification.

3. Antibodies

(1) Monoclonal Antibodies

Western Blot Protocol:

- Prepare protein samples and resolve them using SDS-PAGE.

- Transfer proteins to a nitrocellulose or PVDF membrane.
- Block the membrane with 5% milk in TBST for 1 hour.
- Incubate the membrane with primary monoclonal antibody (1:1000 dilution) overnight at 4°C.
- Wash and incubate with HRP-conjugated secondary antibody (1:5000 dilution).
- Develop using chemiluminescent substrate and visualize bands.

(2) Polyclonal Antibodies

ELISA Protocol for Polyclonal Antibodies:

- Coat 96-well plates with antigen in carbonate buffer and incubate overnight at 4°C.
- Block non-specific sites with 1% BSA.
- Add primary polyclonal antibody (dilution 1:1000) and incubate for 1 hour at 37°C.
- Add secondary antibody conjugated to HRP and incubate for 1 hour.
- Develop with TMB substrate and read absorbance at 450 nm.

(3) Recombinant Antibodies

Immunoprecipitation Protocol:

- Incubate cell lysates with recombinant antibody at 4°C for 2 hours.
- Add protein A/G agarose beads and incubate overnight.

- Wash the beads with lysis buffer to remove non-specific proteins.
- Elute immunoprecipitated proteins and analyze via Western blot or mass spectrometry.

4. Gene and Cell Therapies

(1) Gene Therapy

CRISPR/Cas9 Gene Editing Protocol:

- Design guide RNA targeting the desired gene locus.
- Co-transfect cells with gRNA, Cas9 protein, and donor template (if performing knock-in).
- Incubate cells for 48-72 hours.
- Harvest cells and perform genomic DNA extraction.
- Confirm gene editing via PCR and sequencing.

(2) Cell Therapy

Stem Cell Expansion Protocol:

- Thaw frozen stem cells and plate them in a pre-coated culture dish.
- Culture cells in stem cell-specific medium, changing the medium every 2-3 days.
- Monitor cell morphology and confluency daily.
- When cells reach 80% confluency, passage the cells using trypsinization.

- Harvest stem cells for differentiation or transplantation once the required quantity is achieved.

For further guidance, troubleshooting, or technical support, feel free to contact us via our Support page at csteam-biomed@hotmail.com. We are here to help ensure the success of your experiments.