Protocol for alpha-Tubulin Antibody (Cat. No. 302 203) Sandwich Enzyme-linked Immunosorbent Assay (ELISA) Soluble Proteins

The Enzyme-Linked Immunosorbent Assay (ELISA) is a highly sensitive and specific method used to detect and quantify various biomolecules, including proteins, peptides, antibodies, and hormones. In a **sandwich ELISA**, a capture antibody is first immobilized on a solid surface, typically the wells of a microtiter plate. This antibody selectively binds to the target molecule present in the sample (analyte). Subsequently, a detector antibody—specific to a different epitope on the same target—binds to the captured molecule. Detection is achieved through an enzymatic reaction facilitated by a secondary antibody that is conjugated to an enzyme, producing a measurable signal.

Materials

- **ELISA-Plate:** Maxisorb 96-well plate
- **Microplate shaker**
- **Microplate absorbance reader:** Equipped with filters at 450 nm and a reference wavelength (e.g. 620-650 nm)
- **Protease inhibitors:** (suggested: 1 mM PMSF, 1 µg/ml Aprotinin, 1.5 µM Pepstatin A)

Reagents

- **Capture antibody:** mouse anti-alpha-Tubulin antibody (cat. no. 302 211)
- **Detector antibody**: rabbit anti-alpha-Tubulin antibody (cat. no. 302 203)
- **Secondary detection reagent:** Horseradish peroxidase (HRP) conjugated anti rabbit secondary antibody
- **Coating buffer:** 0.1 M Na-carbonate, pH 9.6 (store 0.5 M stock at -20°C)
- **TBST:** Tris buffered saline with Tween 20 (50 mM Tris, 150 mM NaCl, 0.05% Tween 20), pH 7,2
- **Blocking buffer:** 5% skimmed milk in TBST
- **Substrate solution:** Tetramethylbenzidine (TMB) reagent for development
- **Stop solution:** 0.25 M H_2SO_4 to stop development

Procedure

1. Coat 96-well microplate with 100 µl **capture antibody** (200-400 ng/well) in **coating buffer**. Seal the 96-well microplate and incubate overnight at 4°C.

2. Block the surface with **blocking buffer** for 1 h at RT and 700 rpm on a **microplate shaker**.

3. Wash the plate three times with **TBST** (at least 5 min per wash).

4. Apply antigen or analyt diluted in **blocking buffer** and incubate for 2 h at RT and 700 rpm on a **microplate shaker**.

5. Wash three times with **TBST**.

6. Apply **detector antibody** diluted in **blocking buffer (dilution 1:1000 up to 1:8000)** and incubate for 2 h at RT and 700 rpm on a **microplate shaker**.

7. Wash three times with washing buffer.

8. Incubate with **secondary detection reagent** diluted in **blocking buffer** (diluted according to the manufacturer's recommendations) for 1 h at RT and 700 rpm on a **microplate shaker**.

9. Wash three times with **TBST**.

10. Add 100 µl **substrate solution** for development.

11. Stop the reaction after 5-10 min with 100 µl **stop solution** and measure the absorbance at 450 nm (ref: 620-650 nm).