Everest Biotech Western Blotting Guidelines

- Tris buffered saline (TBS): 20mM Tris, pH7.4 in 150mM NaCl
- Use 12% gel. Voltage is initially 60V for 5 minutes then 200V for 1 hr
- Block transferred PVDF membrane in 3% (w/v) skimmed milk in TBS-T (0.05% v/v Tween 20) for 1hr at room temperature with agitation (can be blocked overnight at 4°C without agitation). We find enhancement of transfer of proteins on PVDF membranes, when compared to nitrocellulose membranes.
- All antibodies are incubated in blocking buffer.
- Incubate Primary antibody for 1 hr at room temperature with agitation, and not overnight. When the reactivity is too strong, dilute further in steps of factor three.
- Wash with TBS-T three times after primary and after secondary antibody, each wash lasting for approx 10 minutes
- We use Everest Biotech Ltd. anti-goat secondary EB2ND-001-HRP (affinity purified and with minimal cross-reactivity with human and rodent serum proteins), range 1:20,000 to 1:40,000) for 1 hr at room temperature with agitation.
- We use ECL-plus (Amersham) rather than ECL, which is more sensitive for detection.
- Where possible include a positive control lysate (e.g.as per datasheet)
- Include a negative control lane – this may be a lysate, or just omission of the Primary antibody.