Western Blot Labeling - general protocol

Datasheets that this protocol applies to – All those where western blots using goat polyclonals on tissue lysates or cell line lysates (human, rat or mouse) are described, except where the data is clearly attributed to an external collaborator.

Starting material: We find enhancement of transfer of proteins on PVDF membranes compared to nitrocellulose membranes.

- The membrane is blocked in 3% (w/v) skimmed milk in TBS-T for 1 hr at room temperature with agitation (can be blocked overnight at 4°C without agitation).
- Primary antibody is incubated in the blocking buffer for 1 hr at room temperature with agitation.
- We use Everest Biotech anti-goat secondary antibody (http://everestbiotech.com/product/rabbit-anti-goat-igg-antibody-HRP-Conjugated/), which is affinity purified and has minimal cross-reactivity with human and rodent serum proteins. It should be diluted at 1:20,000 or higher in the blocking buffer and incubated for 1 hr at room temperature with agitation.
- Wash with TBST three times after primary and after secondary antibody, each wash lasting for 5-10 minutes.
- We use ECL-plus rather than ECL, which is more sensitive for detection. The blots are exposed to X-ray film.

Tris buffered saline (TBS): 20mM Tris, pH7.4 in 150mM NaCl
TBS-T: TBS with 0.05% v/v Tween-20

Important comments:

After transfer on PVDF membrane, the quality of the transfer is monitored by staining in Ponceau red. Areas with visible air bubbles are marked with permanent ink and lanes where an air bubble was trapped at the position of bands of interest should be avoided for analysis. The Ponceau is removed by Tween-containing buffer.

Primary incubation overnight at 4C requires much further dilution of the primary antibody. Still, non-specific background may be expected when incubating overnight.

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