

## **WESTERN BLOT**

### **Trouble shooting guide**

**Problem: *No signals at all, or very poor signals.***

Have you used secondary that is specific to goat antibodies?  
Was the substrate correct for the conjugated enzyme?  
Did you check exposure times and primary dilutions? We recommend primary antibody incubation for 1 hour at room temperature.  
Did you include a positive control lysate as on the product sheet?  
Did you load enough protein in one lane?  
Did you check proper transfer by Ponceau stain?  
Did you incubate at ambient temperatures?  
Did you use fresh antibody, not subject to repeated freeze/thaw cycles?

**Problem: *Too high background.***

Did you dilute primary and secondary far enough?  
Did you block in skimmed milk with 0.05% Tween-20 (TBS or PBS)?  
Did you block long enough before adding the primary?  
Did you wash thoroughly enough in 0.05% Tween-2- (TBS or PBS)?  
Did you wash both between primary and secondary and after secondary?  
Did you use affinity-purified secondary with minimal cross-reactivity to Human, Rat and Mouse serum proteins?

**Problem: *wrong size band.***

Did you include a positive control lysate as on the product sheet?  
Did the band disappear after omitting the primary?  
Did you compare with another antibody against the same target?

**If problems persist, please contact [support@everestbiotech.com](mailto:support@everestbiotech.com)**