WESTERN BLOT Trouble shooting guide

Problem: *No signals at all, or very poor signals.*
Suggestions: Have you used secondary that is specific to goat antibodies?
Did you check that the secondary was in working condition?
Was the substrate correct for the conjugated enzyme?
Did you check exposure times and primary dilutions?
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?

If problems persist, please contact support@everestbiotech.com

Problem: *Too high background.*
Suggestions: 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 in the blocking buffer?
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?
Did you omit the primary to see if background was secondary derived?
Did you incubate primary for 1h at ambient temperature (not overnight)?

If problems persist, please contact support@everestbiotech.com

Problem: *wrong size band.*
Suggestion: 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

Problem: *Product was not tested in physiologically relevant tissue/cell line*
Please contact support@everestbiotech.com with positive suggestions.