ImmunoWay
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Antibody FAQs

- **Can ImmunoWay antibody be used in another technique (WB, ELISA, IHC, IF, IP, ICC) which is not in data sheet?**
- **How much sample should be loaded?**
- **Do you recommend blocking with milk or BSA?**
- **How do I know this antibody is specific for Protein X? Does it cross-react with ProteinX1, X2, X3, X4?**
- **Will this antibody recognize the same target in different species on the data sheet? Does this mean that the other species has not been tested?**
- **Why am I seeing multiple or unexpected bands on a Western with my antibody?**
- **Why am I not seeing any bands on my western when assaying with the purified antibody?**

Can ImmunoWay antibody be used in another technique (WB, ELISA, IHC, IF, IP, ICC) which is not in data sheet?

The package insert for the ImmunoWay antibody specifies the intended use/technique for a ImmunoWay antibody. Any procedure or technique not specified in the package insert must be validated by the user.

How much sample should be loaded?

Cell lysates, membrane and nuclear lysates: load 20 to 30 μg of total protein per well. This may require some optimization depending on the expression level of the protein in the sample you are testing. Purified protein (recombinant or endogenous): load 10 to 100 ng of protein per well.

Do you recommend blocking with milk or BSA?

Unless specifically indicated in the data sheet we recommend blocking with 1X TBS/T with 5% w/v nonfat dry milk. Blocking with BSA or Tween 20 has application for phospho-specific antibodies; in this case Milk should not be used.

How do I know this antibody is specific for Protein X? Does it cross-react with ProteinX1, X2, X3, X4?

Check the specificity for target using about 50 amino acid immunogen region sequence in a protein BLAST homology search. If you are unsure it, please contact technical support for assistance.

Will this antibody recognize the same target in different species on the data sheet? Does this mean that the other species has not been tested?

All of antibodies are generated with peptides designed using the database corresponding to the primary species listed on the ImmunoWay website. These are generally human, mouse or rat. This species was then confirmed with the western blot validation data shown for each antibody in the appropriate species.

If we do not obtain positive results in one species, we do not list that species. However, it is possible that the cell line we test may contain a low level of particular protein or the inductions we used do not work in that particular cell line. Therefore, we suggest you compare the peptide sequence between the species you are studying and the species that the ImmunoWay antibody was raised against. In most cases, ImmunoWay antibodies are raised against the human protein sequence and are highly purified using affinity chromatography. Many times if the sequences are identical or have only 1 or 2 amino acid differences, there is a high possibility the ImmunoWay antibody will recognize your protein.

Why am I seeing multiple or unexpected bands on a Western with my antibody?

It isn't uncommon to see multiple bands even when using affinity-purified antibody. This isn't indicative of a problem with the antibody's specificity. Rather, this typically occurs for one of the following reasons:

1. An excessive amount of lysate loaded onto the gel may cause extra bands. Using decreasing dilutions of lysate will help to determine optimal loading amounts. An increased wash cycle may also alleviate this problem.
2. Lower molecular weight products may be due to protein degradation. We suggest preparing fresh samples by lysing cells/tissues according to our recommended protocol. Compare the signal of your experimental samples with the positive control.
3. Non-specific bands may be due to the secondary antibody. We recommend running a no primary antibody control: verify the appropriate dilution of the secondary detection system, run the Western blot with no primary antibody, and probe only with the secondary detection system.
4. Higher molecular weight products may be a result of post-translational protein modifications, including, but not limited to, glycosylation, myristylation, phosphorylation, and/or ubiquitination.
5. Additionally, higher molecular weight bands may also be a result of protein aggregation that is not resolvable by SDS and boiling.
6. The native protein is a different molecular weight than previously predicted.
7. The antibody is recognizing either cleaved fragments of the native protein at lower molecular weights or aggregated dimers/trimers of the native protein at higher molecular weights.

Why am I not seeing any bands on my western when assaying with the purified antibody?

In cases where no bands are seen (or an antibody doesn't work in a particular assay), these are the most common explanations:

1. This may be due to inadequate transfer of proteins during electroblotting. Try staining the membrane with Ponceau-S immediately after the transfer. This is an effective and non-interfering method to verify proper protein transfer. Consult our recommended Western blotting protocols to optimize these electrophoresis and transfer conditions.
2. The detection enzyme may be inactivated. Sodium azide inactivates horseradish peroxidase (HRP) irreversibly, so do not include sodium azide in any HRP-labeled reagents. Bacterial contamination also diminishes HRP activity. HRP conjugates should be kept bacteria-free, handled with sterile technique and stored under recommended conditions.
3. The polyvinyl wrap from certain sources may quench the signal. We suggest repeating the incubation with chemiluminescence reagents and placing the blot between two pieces of write-on acetate transparency film, and then expose the film.
4. The expression of the protein of interest may be very low. Sensitivity may be increased by performing an immunoprecipitation prior to the Western blot.
5. In cases of very weak protein expression, eliminating Tween during primary antibody incubation may improve antibody binding.
6. To better facilitate Ag-Ab interaction you could incubate primary antibody at 37°C with gentle incubation and further incubate it overnight at 4°C.
7. The peptide sequence corresponds to a non-exposed region of the native protein.
8. The protein's conformation in the peptide region differs enough that the antibody has trouble recognizing the native protein.
9. The target protein isn't present in the sample.