

## ClearBand HRP Conjugated Goat anti-rabbit IgG Antibody Troubleshooting Guide

500 µL

Cat No: HRP5

Use this guide for common Western blot and ELISA detection problems with ClearBand HRP Conjugated Goat anti-rabbit IgG Antibody. The affinity-purified goat polyclonal secondary antibody is HRP-conjugated, reacts with whole-molecule rabbit IgG and rabbit immunoglobulin light chains, and is supplied in PBS containing 50% glycerol and 0.05% ProClin 300.

### Before You Start - Quick Checks

1. Store the antibody undiluted at -20°C and protect it from prolonged exposure to light.
2. Confirm that the primary antibody is rabbit-derived before using this anti-rabbit secondary antibody.
3. Do not assume a universal working dilution; the datasheet does not specify one, so optimize the secondary-antibody concentration for the assay system.
4. Use clean, consistent blocking and wash conditions and an HRP-compatible detection substrate.

| Problem                                                    | Most likely cause                                                                                                                      | Recommended action                                                                                                                                                 |
|------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>No or very weak signal</b>                              | The secondary antibody may not match the host species of the primary antibody, or the working concentration may be too low.            | Confirm that the primary antibody is rabbit-derived, then optimize the secondary-antibody concentration and verify the HRP detection substrate and imaging system. |
| <b>High background across the membrane or plate</b>        | The secondary antibody may be too concentrated, blocking may be insufficient, or washing may be inadequate.                            | Titrate the secondary antibody to a lower working concentration and optimize blocking and wash steps while keeping the primary-antibody conditions unchanged.      |
| <b>Multiple nonspecific bands</b>                          | Excess secondary antibody or species cross-reactivity may contribute to nonspecific detection.                                         | Reduce the secondary-antibody concentration and confirm that the sample system does not contain immunoglobulins that can be recognized nonspecifically.            |
| <b>Signal appears with the wrong primary-antibody host</b> | The antibody is directed against rabbit IgG and can also recognize rabbit immunoglobulin light chains.                                 | Use the secondary antibody only with the intended rabbit-primary workflow unless cross-reactivity has been specifically validated.                                 |
| <b>Signal varies between experiments</b>                   | Working dilution, incubation, washing, or exposure conditions may not be standardized.                                                 | Use the same antibody dilution, incubation time, wash conditions, substrate preparation, and imaging settings across experiments.                                  |
| <b>Signal decreases after storage</b>                      | The antibody may have been exposed to unsuitable temperature, repeated handling, or prolonged light.                                   | Keep the antibody undiluted at -20°C and protected from prolonged light exposure; minimize unnecessary warming and handling.                                       |
| <b>Strong signal but poor signal-to-noise ratio</b>        | The antibody/substrate combination may be too sensitive for the current exposure or secondary concentration.                           | Reduce secondary-antibody concentration and/or shorten imaging exposure while maintaining adequate washing.                                                        |
| <b>Unexpected signal in non-rabbit systems</b>             | The datasheet notes minimal cross-reaction with mouse, human and rat immunoglobulins, but cross-reaction with other species may occur. | Interpret unexpected species signals cautiously and validate cross-reactivity experimentally for the sample system being used.                                     |

Still having trouble? Recheck primary-antibody host species, secondary-antibody titration, blocking/washing, HRP substrate preparation, exposure settings, and -20°C light-protected storage before changing the primary antibody.