

# Living Colors<sup>®</sup> Peptide Antibody Conjugates

## Protocol-at-a-Glance

(PT3355-2)

### Solutions Required

- **Phosphate-buffered saline** (10 mM PBS, 144 mM NaCl; pH 7.4)
- **Wash buffer** (PBS with 0.2% Tween-20)
- **Blocking buffer** (PBS with 0.2% Tween-20, and 5% (w/v) nonfat dry milk)

### General Western Blot Procedure

1. Rinse the membrane twice with PBS for 5 min each time. Do not allow the membrane to dry.
2. Incubate the membrane in blocking buffer at room temperature for 1 hr. Alternatively, this incubation may be done at 4°C overnight.
3. Dilute the Living Colors Peptide Antibody Conjugate to the appropriate dilution with blocking buffer.

**Note:** For both the Alkaline Phosphatase (AP) and Horseradish Peroxidase (HRP) conjugates, we recommend starting at a 1:500 dilution for high GFP-expression levels, and a dilution of 1:200 for low GFP-expression levels. The optimal dilution of the antibody may vary with individual systems and must also be determined empirically.

4. Incubate the membrane with the diluted Living Colors Peptide Antibody Conjugate at room temperature for 2 hr or at 4°C overnight.

**Note:** The optimal incubation times may vary with individual systems and must be determined empirically.

5. Wash the membrane a minimum of 4 times in wash buffer for 5 min each time. Additional washes may reduce background signals which are produced by nonspecific binding of the antibody to the membrane.
6. Proceed with the appropriate detection methods for AP or HRP.

### References

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3. Kain, S. R. & Henry, H. L. (1990) Quantitation of proteins bound to polyvinylidene difluoride membranes by elution of Comassie Brilliant Blue R-250. *Anal. Biochem.* **189**:169–172.
4. Towbin, H., Staehelin, T. & Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350–4356.

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