



**Title:** *Western Blot Protocol*

No: RTLP-GLP-LJ6

Location:  
*Old CCRC Tripp Lab*

Approval Date:  
10 September 2004

Supersedes Date:

**Materials:**

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- |  |  |                               |                  |
|--|--|-------------------------------|------------------|
| •Lab coat  | •Tris HCL  | •ECF substrate                | •Pipettes        |
| •Gloves  | •NaCl  | •Typhoon 9210                 | •Pipetteman      |
| •2x Laemmli buffer   | •Tween-20  | Laser Scanner                 | •Pipette Aid     |
| • $\alpha$ -mouse IgG (H+L)<br>conjugated to alkaline<br>phosphatase | •4-20% gradient<br>Criterion<br>polyacrylamide<br>Tris-HCL gel | •Criterion SDS-PAGE<br>system | •Pipetteman tips |
| •antigen-specific mouse<br>IgG                                       | •PVDF Membrane   |                               |                  |
|  | •Powdered Milk   |                               |                  |

**Procedure:**

1. Mix protein sample with an equal volume of 2X Laemmli buffer in 1.5ml tube, boil 5 minutes and separate by SDS-PAGE on a 4-20% gradient Criterion polyacrylamide Tris-HCL gel (BioRad, Hercules, CA).
2. Electrotransfer proteins onto a PVDF membrane (BioRad, Hercules, CA) for 2 hours at 4°C under standard conditions.
3. Block membrane with 5% powdered milk in wash buffer (50mM Tris, 150 mM NaCl, 0.05% Tween 20) for 1hour at room temperature, and then wash 1X for 15 minutes in washing buffer at room temperature.
4. Dilute antigen-specific mouse IgG into wash buffer and incubate with the membrane for 1 hour at room temperature.
5. Wash the membrane in washing buffer at room temperature 1X for 15 minutes, followed by 2X for 5 minutes each.

6. Dilute anti-mouse IgG(H+L) conjugated to alkaline phosphatase (Pierce, Rockford, IL) into washing buffer and incubate with the membrane for 1 hour at room temperature.
7. Wash membrane in washing buffer at room temperature 1X for 15 minutes, 2X for 5 minutes each.
8. Pipette ECF substrate (Amersham Biosciences, Piscataway, NJ) onto the surface of the membrane and allow the reaction to proceed for 15 minutes at room temperature.
9. Remove excess substrate and allow the membrane to completely air dry at room temperature.
10. Assay membrane fluorescence on the Typhoon 9210 Laser Scanner (Amersham Biosciences, Piscataway, NJ).