Western Blot Analysis—Phosphotyrosine
AfCS Procedure Protocol PP00000006
Version 1, 01/17/01

This procedure is the final step in a 3-step process of Western immunoblotting for
detection of protein phosphorylation on tyrosine residues. The first step is sodium
dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to resolve a mixture of
proteins by size (AfCS Protocol SDS-PAGE—Invitrogen Mini-Cell, PP00000002 or
SDS-PAGE—Bio-Rad Criterion Cell, PP00000003). The second step in the process is
the transfer of resolved proteins from the gel to a membrane support (i.e., nitrocellulose
or polyvinylidene difluoride [PVDF]) via electroelution (AfCS Protocol Protein Transfer—
Invitrogen XCell SureLock Mini-Cell, PP00000004 or Protein Transfer—Bio-Rad
Criterion Blotter, PP00000005). The product of the second step is referred to as the
blot. This final step involves processing of the blot for detection of phosphotyrosine with
an antibody, the product of which is the final Western immunoblot.

Procedures
Unless otherwise stated, all of the following incubations and washes are conducted at
room temperature with gentle shaking using an oscillating platform shaker. Volumes are
specified for 8 x 8 cm Invitrogen gel blots (should be doubled for 8.7 x 13.3 cm Bio-Rad
Criterion gels).

1. Wash blot twice, each time for 5 min, with 25 ml Tris-buffered saline with Tween
   20, 0.1% (0.1% TBST).
2. Incubate blot for 1 hr in 25 ml of bovine serum albumin, 5% in Tris-buffered saline
   Tween 20, 0.1% (5% BSA/0.1% TBST).
3. Quickly rinse blot two times with 15 ml 0.1% TBST.
4. Incubate blot for 2 hr at room temperature in 10 ml p-Tyr (PY99) solution. Note: the
   p-Tyr (PY99) antibody is conjugated to horseradish peroxidase; thus, no secondary
   antibody is necessary.
5. Wash blot three times, for 10 min each, with 0.1% TBST.
6. Prepare fresh solution of ECL Plus Western Blotting Detection Reagents (ECL
   Plus).
7. Incubate blot in ECL Plus for 5 min, shaking vigorously.
8. Lift blot with blunt forceps and drain briefly by touching one end on paper towel.
9. Place damp blot in transparent sheet protector, being careful not to trap air
   bubbles.
10. Create image with Molecular Dynamics Storm 860 gel and blot analysis system.

Reagents and Materials
Tris-buffered saline with Tween 20, 0.1% (0.1% TBST), 4 L: AfCS Solution Protocol ID
PS00000064

Bovine serum albumin, 5% in Tris-buffered saline Tween 20, 0.1% (5% BSA/0.1%
TBST), 500 ml: AfCS Solution Protocol ID PS00000020

p-Tyr (PY99) solution (p-Tyr[PY99]), 10 ml: AfCS Solution Protocol ID PS00000049
Transparent sheet protector: C-Line Products; catalog no. 00010

Molecular Dynamics Storm 860 gel and blot analysis system: Amersham Pharmacia Biotech; catalog no. 860PC
  Includes 520 nm long-pass filter for blue-excited fluorescence to detect chemifluorescence emitted by ECL Plus

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