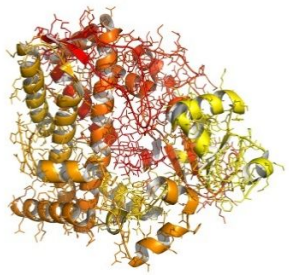

CYP450-GP
2870 Scott Street
Suite 103
Vista, CA 92081
Phone 760.295.7426
Fax 760.418.6472
support@cyp450-gp.com

WESTERN BLOTTING PROTOCOL

1. For full size (15 x 15 cm) blots, add **100 – 150 µg** of CYP450-GP primary P450 antibody (e.g., rabbit anti-human CYP3A4 or anti-human CYP2C9) to 15-20 ml of blocking solution (e.g., 5% non-fat dry milk in PBS; i.e., Blotto). This corresponds to 100 -150 µl of the 1.0 mg IgG/ml solutions reconstituted in PBS/glycerol as described for each antibody.

2. After incubating overnight at room temperature in a covered tray, discard primary P450 antibody solutions. Extensively wash the blot with blocking solution to remove traces of unbound 1°AB, and then incubate with an **anti-rabbit** IgG conjugate of your choice (e.g. goat anti-rabbit IgG-alkaline phosphatase).

3. We use a goat anti-rabbit IgG-Biotin X conjugate, followed by a complex of strepavidin plus HRP-Biotin X, and subsequent staining with 4-chloro-1-naphthol/hydrogen peroxide. Our immunochemical development system allows good visualization of CYP3A4 and CYP2A6 with loads of 5-10 µg microsomal protein applied to the original polyacrylamide gel. However, an anti-rabbit IgG-alkaline phosphatase conjugate of high specific activity with BCIP/NBT colorimetric staining should be at least as sensitive.