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## Anti-PARP Antibodies

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### Protocol

1. HL-60 cells are prepared from exponentially growing cells either induced for apoptosis with etoposide (Cat. No. 341205) or uninduced. Cells are washed once with PBS, suspended at  $\sim 4 \times 10^6$  cells/ml in sample buffer (62.5 mM Tris-HCl, pH 6.8, 6 M urea, 10% glycerol, 2% SDS, 0.00125% bromophenol blue, 5%  $\beta$ -mercaptoethanol). Cells are sonicated for 15 seconds and incubated at 65°C for 15 minutes.

*Note: The purpose of the urea and sonication step is to effectively dissociate PARP/DNA interactions. For preparing PARP electrophoresis samples from tissue, see Shah, G.M., et al. 1995. Anal. Biochem. **227**, 1 or Simonin, F., et al. 1991. Anal. Biochem. **195**, 226.*

2. Run SDS-PAGE using 20  $\mu$ l of control or induced HL-60 extract.
3. Transfer proteins to nitrocellulose blotting membrane.
4. Block with 5% non-fat dry milk in TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween<sup>®</sup> 20) for 1 hour at room temperature.
5. Incubate nitrocellulose with the appropriate dilution of anti-PARP antibody in TBST with 5% non-fat dry milk for 2.5 hours at room temperature.
6. Wash membrane 3 x with TBST, 10 minutes/wash.
7. Incubate nitrocellulose with secondary antibody in TBST with 5% milk, using either anti-mouse or anti-rabbit alkaline phosphatase conjugate, as appropriate, for 1 hour at room temperature.
8. Wash membrane 3x in TBST, 10 minutes per wash. Rinse briefly with TBS (TBST without the Tween<sup>®</sup> 20).
9. Incubate with BCIP/NBT color development reagent for  $\sim 5$  minutes until bands reach desired intensity. Rinse with TBS plus 20 mM EDTA to stop color development. *Note: color development times may vary.*

**These procedures are intended only as a guide. Optimal concentrations of primary antibody and experimental conditions must be determined by the individual user.**

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