BlotCycler™
by PRECISION BIOSYSTEMS

APPLICATION NOTE

Improved Specificity of Western Blot Following Processing at 4°C Using BlotCycler

Courtesy: Laura Marlow, Mayo Clinic, Jacksonville, FL

A comparison of western blot assays obtained by performing washing, blocking and antibody incubation partially and entirely at 4°C:

Western blots with cell extract from the clear cell renal carcinoma (ccRCC) cell line were incubated with the rabbit polyclonal anti PARP antibody (Cell Signal, Cat #9542).

The steps for washing, blocking and antibody incubation are as follows: Membranes were blocked with TBST 5% dry milk for 1 hour, incubated with primary antibody overnight, washed 3X15 minutes with TBST, incubated with the anti-rabbit secondary antibody (Jackson Immunochemicals, 1:4000 dilution) for 1 hour, and washed 3X15 minutes TBST. The bands were visualized using a chemiluminescence substrate.

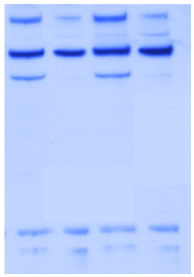
Two procedures for performing the above mentioned steps were compared. The manual procedure involved incubation of the primary antibody at 4°C while the rest of the procedure was performed at room temperature. The automated procedure involved performing all the processing steps 4°C using the using ***BlotCycler*** automated system.

When the blot was processed using the manual procedure, multiple non-specific bands were observed, see figure A below. For the blot processed with the automated procedure at 4°, only the bands representing the uncleaved PARP protein (113 kD) as well as the C-terminal and N-terminal cleaved PARP fragments (89 kD and 24 kD respectively) were detected, see figure B below.

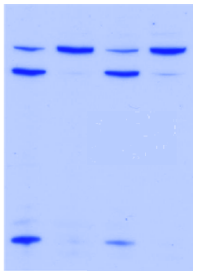
Conclusion:

These data suggest the importance of how blocking and washing steps are performed, as well as the temperature of blot washing and antibody incubation. Hands-free western blot processing using ***BlotCycler*** enables researchers to optimize shaking, washing, and antibody incubation condition during western blot processing to eliminate nonspecific bands and generate high quality results.

A. Manual procedure: Primary antibody was incubated at 4°C, and then membrane was processed at room temperature. As seen here (at right), multiple non-specific bands are visible.



B. *BlotCycler* automated procedure: All the steps in the protocol, including incubation, were performed at 4°C – virtually ***no non-specific bands*** are visible.



About Precision Biosystems

BlotCycler was developed and manufactured by Precision Biosystems, a Massachusetts-based company that provides solutions to help researchers automate difficult, time-consuming tasks in their laboratories.

www.blotcycler.com 1-888-490-4443 E: hello@blotcycler.com 241 Francis Avenue Mansfield, MA 02048

precision BIOSYSTEMS