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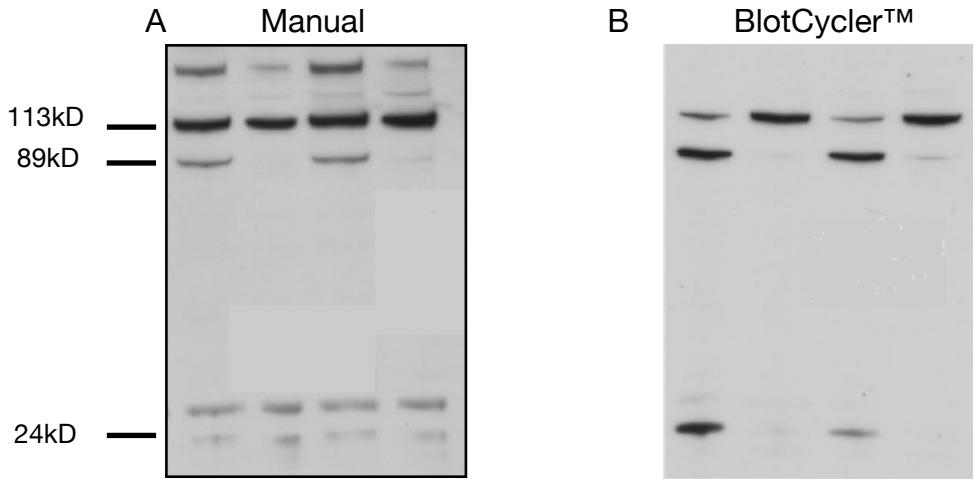
Improved Specificity of Western Blot Following Processing at 4°C Using BlotCycler



Western blot processed by BlotCycler™ as compared to the traditional manual procedure. 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) using the traditional manual procedure (A) or BlotCycler™ (B). The membranes were processed as follows: 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.

aFor the manual procedure, incubation with the primary antibody was carried out at 4°C while the rest of the procedure was performed at room temperature.

Processing using BlotCycler™ was done entirely at 4°C. When the blot was processed using the traditional manual method (A), multiple non-specific bands were observed. In contrast, the blot processed with BlotCycler™ (B) were detected 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).

These data suggest the importance of how blocking and washing steps are performed, as well as the temperature of blot washing and antibody incubation. Handsfree 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..

Courtesy: Laura Marlow, Mayo Clinic, Jacksonville, FL

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