

AdvanBlock™-Chemi

Antibody-antigen signal enhancing and blocking solution for chemiluminescent Western blots

Evaluation of the stability of an anti-phospho STAT3 polyclonal antibody diluted in AdvanBlock™-Chemi compared to 5% Milk-PBST + 0.05% NaN₃

Typically, it is not advised to reuse your diluted antibody solution as it may result in variability and loss of signal. However, it is understandable that there are circumstances that may merit reuse such as cost and availability. The high cost of commercially available primary antibodies drives up the cost of the Western blot, a common method for protein analysis.

The biggest concern when reusing your diluted antibody solution is microbial contamination. AdvanBlock™-Chemi contains a biocide which immediately kills microbes as a result of its ability to

quickly penetrate cell membranes and inhibit specific enzymes crucial to cellular respiration. In order to inhibit growth in the 5% Milk-PBST solution, sodium azide was added. Even with the addition of sodium azide, the 5% Milk-PBST solution will eventually go bad as the complex matrix is not a stabilized solution.

Caution should always be taken when deciding to reuse your antibody solution as each antibody has a unique affinity and stability. High quality antibodies that have been affinity purified are typically expected to be more stable, allowing for reuse.

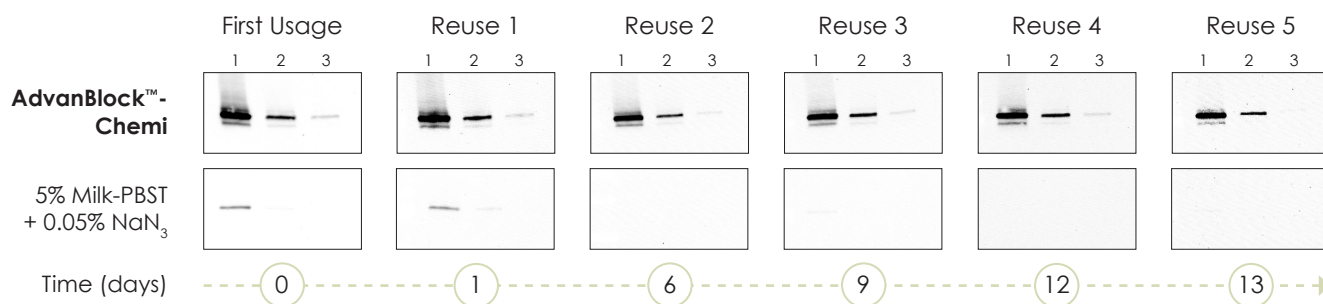


Figure 1. Anti-phospho STAT3 demonstrates increased stability in AdvanBlock™-Chemi compared to 5% Milk-PBST + 0.05% NaN₃. (A) 5-fold serial dilutions of IFN α -treated HeLa lysate (5, 1 and 0.2 μ g) were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The blots were blocked with AdvanBlock™-Chemi or 5% Milk PBST before incubation with anti-phospho STAT-3 (Cell Signaling Technology #9145S). Sodium azide was added to the milk solution used to dilute the primary antibody to prevent microbial growth. After the primary antibody incubation step was complete, each blocking solution was transferred to a sterile 15 mL conical tube and stored at 4°C. Signal was detected with WesternBright® ECL. In addition to the enhancement of specific signal, the antibody diluted in AdvanBlock™-Chemi shows increased stability demonstrated by the reuse of the antibody solution more than 5 times over the course of two weeks.

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