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Effects of Specific Blocking Buffers on Histone H2B Antibodies

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Mammalian histones have been known to be one of the most highly alkaline proteins found in the nuclei. Their main function is to organize DNA into chromosomes and regulate transcription. An example of a histone would be Histone H2B, which this project focuses heavily on. Histone H2B has been seen to bind poorly to antibodies and result in a weak signal. The goal of this project is to test a multitude of blocking buffers, in triplicate, in order to find a blocking buffer that can obtain the strongest signal from the antibodies being tested. Through the use of protein gel electrophoresis and western blotting procedures, the different blocking buffers could be compared. The blocking buffers used for these experiments consisted of 5% nonfat dry milk in Tris-buffered saline (otherwise known as BLOTTO control), 2.5% Milk + 2.5% Hemoglobin, 7% Hemoglobin, 1% Milk +4 % Hemoglobin, and 5% Hemoglobin. After conducting the experiments, it was concluded that hemoglobin is not a useful blocking buffer.