The Impact of Blocking Buffers on Brain Immunohistochemistry

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Abstract

Motivation: The identification of proteins in tissue samples is important for knowing whether a protein is present
Personal Objectives: Find the optimal blocking buffer composition
Results: There were no statistical differences within our experiment

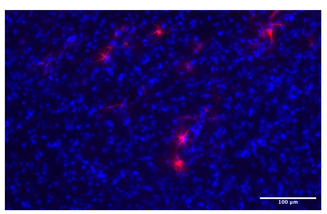
Background

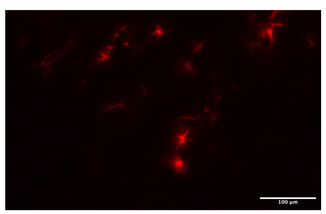
Immunohistochemistry (IHC) is a tool that labels proteins—making them fluorescent under the microscope

- An analytical and diagnostic tool
  - Can be used to identify tumor markers in cancer cells
- With the naked eye, one cannot see certain details in tissue samples such as proteins or differentiation between certain cells

How Immunohistochemistry works:

- Antibodies target and bind to certain proteins
- Primary antibodies bind to target proteins
- Secondary antibodies bind to primary antibodies, omitting fluorescence
- Blocking buffers stop unspecific binding of the antibodies, which can lead to disruption in the results
  - Blocking buffers have two components, one to open the cell membrane (Triton), and one to block unspecific binding (Donkey Serum and Bovine Serum Albumin (BSA))





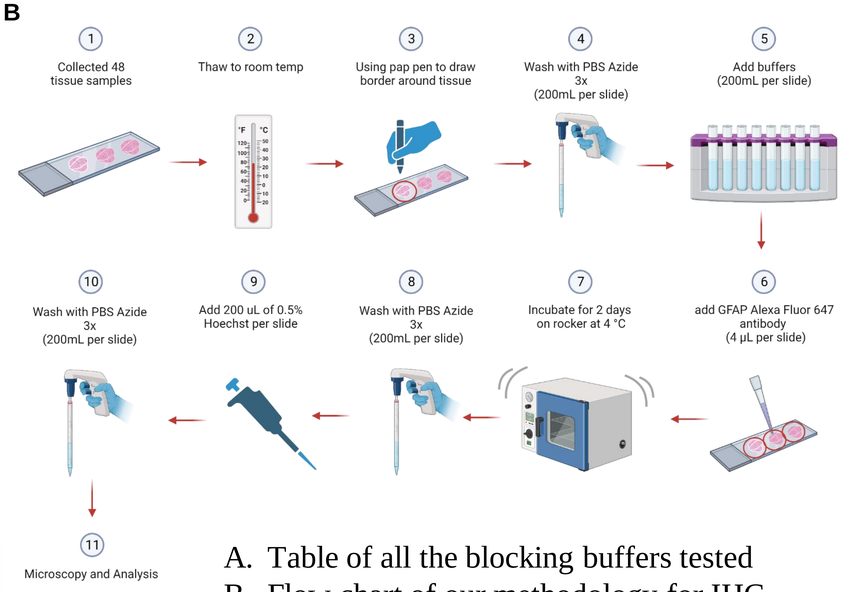
Mouse Brain Sections: Nuclei (Blue), Astrocytes (Red), 20x, Scale Bar (100µm)

Experimental Methods

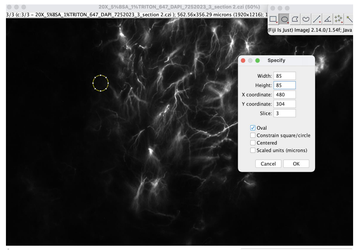
A

Blocking Protein (%)	Triton Amount (%)
Donkey 2%	1%
Donkey 5%	1%
BSA 2%	1%
BSA 5%	1%
BSA 5%	0.7%
BSA 5%	0.4%
BSA 5%	0%

B



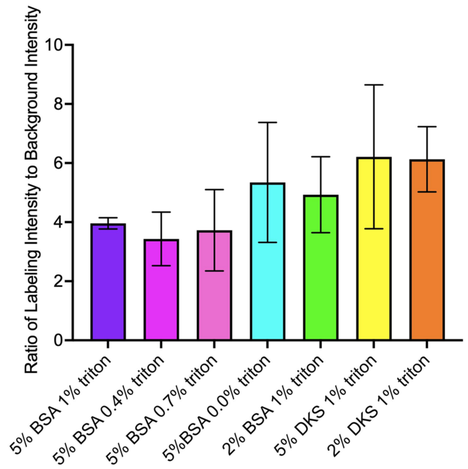
C



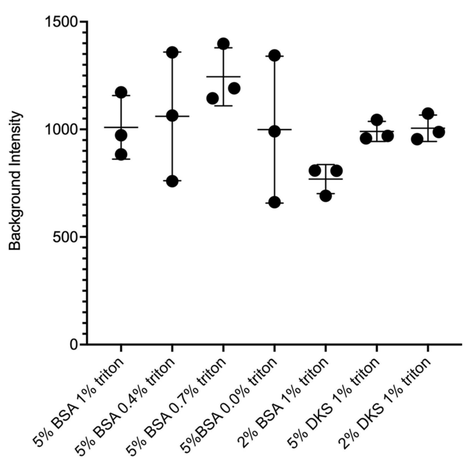
A. Table of all the blocking buffers tested
B. Flow chart of our methodology for IHC
C. Representative image of image analysis using Image J. Mean intensity was collected to compare background versus labeled intensities

Results

D



E

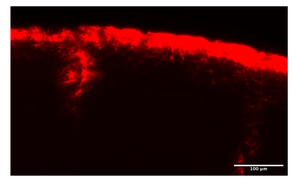


D. Compared background and labeled areas. No differences were found between the buffers
E. Background areas were compared. No differences were found, but 5% Donkey Serum, 2% Donkey Serum, and 2% BSA showed smaller standard deviations

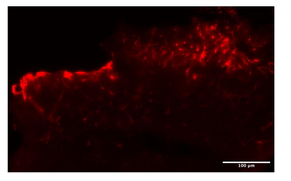
Conclusion and Future Steps

- No statistical differences in the blocking buffers tested
- Due to lack of statistical significance, BSA can be used since it is cheaper

- Reduce Variation
  - Obtain more data points
  - Use similar spots in the brain
- Further optimize the protocol
  - Explore buffer concentrations from 0-2% BSA and 0-0.4% Triton



5% BSA 1% Triton



5% BSA 0% Triton

References

Siracusa, R., Fusco, R., & Cuzzocrea, S. (2019, August 30). *Astrocytes: Role and functions in brain pathologies*. Frontiers. https://www.frontiersin.org/articles/10.3389/fphar.2019.01114/full

Duraiyan, J., Govindarajan, R., Kaliyappan, K., & Palanisamy, M. (2012, August). *Applications of immunohistochemistry*. Journal of pharmacy & bioallied sciences. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3467869/

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