Dec 05, 2019

Immunofluorescent Staining of Foxp3 in Frozen Sections V.3

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1 Works for me dx.doi.org/10.17504/protocols.io.97ch9iw

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ABSTRACT

T regulatory cells (also known as Tregs or Regulatory T cells) are essential cells in the immune system that suppress immune responses of other cells, designed to limit excessive reactions and prevent autoimmunity. Tregs are characterized by the expression of CD4, CD25, and FOXP3, while lacking CD127. CD4+FOXP3+ regulatory T cells have been referred to as "naturally-occurring" regulatory T cells to distinguish them from "suppressor" T cell populations that are generated in vitro. While other variants of suppressive T cells do exist, such as CD8 suppressor cells, Th3 and Tr-1 cells, Tregs are classically defined as CD4+CD25+FOXP3+ cells. Here, we provide a protocol for immunofluorescent staining of FOXP3 in frozen sections, adapted from a protocol from Dr. Matthias Hardtke-Wolenski, Medizinische Hochschule Hannover. This protocol has been successfully used on mouse spleen, liver, and skin sections.

EXTERNAL LINK

https://www.biolegend.com/protocols/immunofluorescent-staining-of-foxp3-in-frozen-sections/4254/

GUIDELINES

General Tips and FAQ:

Can I use GFP expressing cells with Alexa Fluor® 488 or FITC?

- No, due to spectral emission overlap you will not be able to distinguish between GFP and AF488/FITC.

Are fluors such as BV570™, BV605™, BV650™, BV711™, BV785™, PE/Cy7, PE/Cy5, APC/Cy7, PE, APC, PerCP, and FITC good for IF?

- These dyes due to their vulnerability to photo bleaching these are not recommended for IF. Alexa Fluor® and Dylight® fluors, as well as BV421™ and BV510™, are good alternatives for IF application.

MATERIALS TEXT

Antibody Dilution Buffer:

- 1% BSA (blocking & stabilizer)
- 0.1% cold fish skin gelatin (blocking)
- 0.5% Triton X-100 (penetration enhancer)
- 0.05% sodium azide (preservative) in TBS Mix well and store at 4°C

1 Thaw frozen sections in a sealed environment to avoid damage by frozen water crystals (Approximately 1 hour).

2 Fix for 5 minutes in acetone (-20°C). Note: Do not use more often than 5 times.

3 Dry for 10 minutes at room temperature.

4 Incubate sections in TBS-T (50 mM Tris, 150 mM NaCl, adjust pH with HCl to 7.6 with 0.05% Tween 20) for 15 minutes.

5 Pretreatment: Perform antigen retrieval or enzyme digestion if needed.

6 Wash twice for 2 minutes each with TBS-T.

7 Optional: staining of surface marker can be performed before "Cleaning" step.

8 Cleaning: Incubate sections with 1% Triton X-100 diluted in TBS for 30 minutes at room temperature. This step will help to reduce background staining (longer incubation may be more effective, especially for sections thicker than 10 µm).

9 Normal Serum Blocking: Without washing, incubate sections directly with 5% normal mouse/rat/rabbit serum blocking solution for 30 minutes at room temperature.Note: Normal serum should be the same species of which the secondary antibody is raised.

10 Wash three times for 2 minutes each with TBS-T.

11 Endogenous Peroxidase Blocking: Incubate sections with 3% H2O2 in TBS for 10 minutes to block endogenous peroxidise (Recommended for liver sections, but optional for other tissues).

12 Wash 3 times for 2 minutes with TBS-T.

13 Primary Antibody: Incubate sections with primary antibody at its optimal dilution (Mouse FOXP3 Alexa Fluor® 647, clone MF-14, cat # 126408) in "antibody dilution buffer" for 30-60 minutes at room temperature.Note: Using a purified unconjugated antibody along with a secondary reagent may improve staining due to signal amplification.

14 Wash twice for 2 minutes with TBS-T.

15 Counterstain twice for 3 minutes each with DAPI.

16 Wash twice for 2 minutes with TBS-T.

17 Coverslip with Mowiol or mounting medium.