Immunofluorescent Staining of Foxp3 in Frozen Sections

Kelsey Miller

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.

Citation: Kelsey Miller Immunofluorescent Staining of Foxp3 in Frozen Sections. **protocols.io** dx.doi.org/10.17504/protocols.io.e3pbgmn
Published: 06 Jun 2016

Guidelines

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 4ºC.

Protocol

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

DURATION
01:00:00

Step 2.

Fix for 5 minutes in acetone (-20°C)

(note: do not use more often than 5 times).

DURATION
00:05:00

Step 3.

Dry for 10 minutes at room temperature.

DURATION
00:10:00

Step 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.

DURATION
00:15:00

Step 5.

Pretreatment: Perform antigen retrieval or enzyme digestion if needed.

Step 6.

Wash twice for 2 minutes each with TBS-T

Step 7.

(Optional: staining of surface marker can be performed before “Cleaning” step)

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 incubationmay be more effective, especially for sections thicker than 10 µm).

DURATION
00:30:00

Step 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.

Step 10.

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

Step 11.

Endogenous Peroxidase Blocking: Incubate sections with 3% H_2O_2 in TBS for 10 minutes to block endogenous peroxidise. (Recommended for liver sections, but optional for other tissues.)

DURATION
00:10:00

Step 12.

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

Step 13.

Primary Antibody: Incubate sections with primary antibody at its optimaldilution (Mouse Foxp3-Alexa Fluor® 647, clone MF-14, cat # 126408) in “antibodydilution buffer“ for 30-60 minutes at room temperature.

Note: Using a purifiedunconjugated antibody along with a secondary reagent may improve staining due tosignal amplifcation.

Step 14.
Wash twice for 2 minutes with TBS-T.

Step 15.
Counterstain twice for 3 minutes each with DAPI

Step 16.
Wash twice for 2 minutes with TBS-T.

Step 17.
Coverslip with Mowiol or mounting medium.