# IL-4 *in situ* staining

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**Method Article**

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# Abstract

# Introduction

The prototypic Th2 cytokine IL-4 is essential for the development of Th2 responses, as it promotes the differentiation of T helper cells into type 2 effector cells and inhibits type 1 responses. The expression of this important cytokine is very tightly regulated, and IL-4 is also at very low quantities compared to other cytokines \(it is expressed 1000-fold lower than its Th1 counterpart, IFN-gamma), making its detection extremely difficult[1]. In an attempt to examine IL-4 expression _in vivo_, recent studies have employed IL-4 reporter knockin mice, where an IL-4 promoter drives the expression of GFP to identify IL-4 expressing cells, and have described IL-4 expression by basophils and eosinophils, as well circulating T cells[2-5]. Although quite useful, potential caveats with this system are that expression of the GFP may not be similar to IL-4 protein, or even reflect the cytokine's mRNA levels, as the GFP is processed differently by the cell and has a much longer half-life. An additional shortcoming of this approach is that it is not readily useful for _in situ_ imaging. To directly examine IL-4 protein expression patterns _in situ_, we developed a new methodology, where two antibodies recognizing distinct epitopes of the IL-4 proteins were used in tandem. These antibodies were conjugated to the same fluorochrome, resulting in amplification of the fluorescent IL-4 signal enough to be detected by conventional fluorescent microscopy. Additional antibodies conjugated to different fluorochromes can be added to the staining cocktail, to identify distinct leukocyte populations, etc, and these images merged with the IL-4 images.

# Reagents

Antibodies: IL-4: 11B11-Alexa488 \(BD PharMingen, San Diego, CA), BDV6-24G2-Alexa488 \(Caltag, Burlingame, CA). Isotype control: Rat IgG1-Alexa488 isotype control \(BD PharMingen, San Diego, CA). Solutions: Staining solution: 10% rat sera, 0.1% BSA, PBS. Detergent wash solution: 0.5% Tween-20 in PBS. Washing solutions: PBS, dH2O. Fluoromount G \(\(Southern Biotechnology Associates, Birmingham, AL) ANTIBODY COCKTAILS: Dilute antibodies in staining solution. For IL-4 cocktail, use 20µg/ml of 11B11-Alexa488 \(BD PharMingen, San Diego, CA), and 10µg/ml BDV6-24G2-Alexa488 \(Caltag, Burlingame, CA). For the isotype control cocktail, use 30µg/ml of Rat IgG1-Alexa488 isotype \(BD PharMingen, San Diego, CA).

# Procedure

1. Obtain serial frozen tissue sections 4-8µm thick on glass microscope slides. 2. Fix slides in chilled acetone for 10 minutes in a crystal slide jar. For all other washes, plastic slide incubation chambers are sufficient. 3. Air dry slides, and outline tissue section with a hydrophobic marker. 4. Rehydrate the tissue but submerging the slide in PBS for 10-30 seconds. 5. Apply antibody cocktail. Slides should be placed tissue side up in a dark box humidified with a wet paper towel. The antibody cocktail should cover the entire region outlined by the hydrophobic marker. For every staining run, a serial tissue section should be

stained with the isotype control cocktail. Incubate at room temperature for 45 minutes. 6. After this incubation, blot off excess staining cocktail carefully avoiding touching the tissue itself, and rinse the slide in PBS with gentle, manual shaking for 10-30 seconds. 7. Wash slides in detergent wash solution with gentle manual shaking for 10-30 seconds. Repeat this wash step once in fresh detergent wash solution. 8. Wash slides in PBS with gentle, manual shaking for 10-30 seconds. 9. Wash slides in $dH_2O$ with gentle, manual shaking for 10-30 seconds. 10. Add a drop of Fluoromount G to the tissue section. Avoid bubbles by administering the Fluoromount G with a transfer pipet. Carefully apply a coverslip, so the Fluoromount G is evenly distributed underneath without bubbles. 11. The stained slides can be imaged immediately. However, they can be stored, tissue side up, in a dark box at 4˚C. 12. CRITICAL STEP. For imaging the slides, the Alexa 488 fluors can be visualized using FITC excitation and emission filters. It is important to use identical exposure times and image normalization settings for the IL-4 and isotype images. Specific conditions depend on the fluorescent microscopy equipment available. IL-4 protein staining \(shown below, panel C) is punctuate, while minimal staining is detected in a serial section stained with isotype control antibodies \(panel D).

## Timing

1-2 hours

## Critical Steps

12

## References

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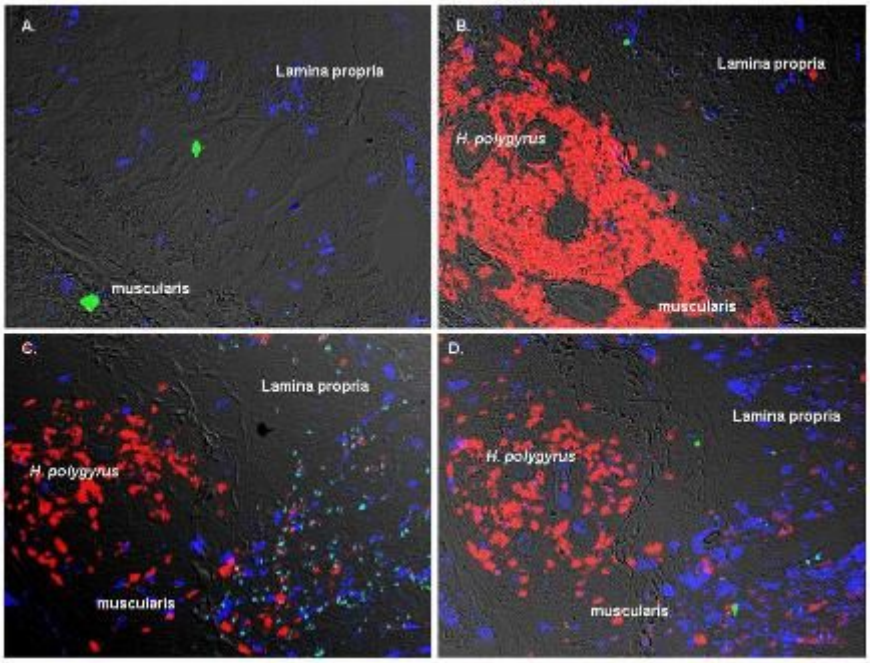
## Figures




## Figure 1

IL-4 _in situ_ staining detection IL-4 protein at the host:parasite interface 4 days following challenge with the gastrointestinal nematode parasite, _Heligmosmoides polygyrus_. *A.* Untreated small intestinal tissue show no infiltration of Gr1+ cells (red), no IL-4 protein (green), and resident CD4+ T cells (blue). *B.* Four days following H. polygyrus primary infection, Gr1+ neutrophils (red) accumulate adjacent to the parasite,