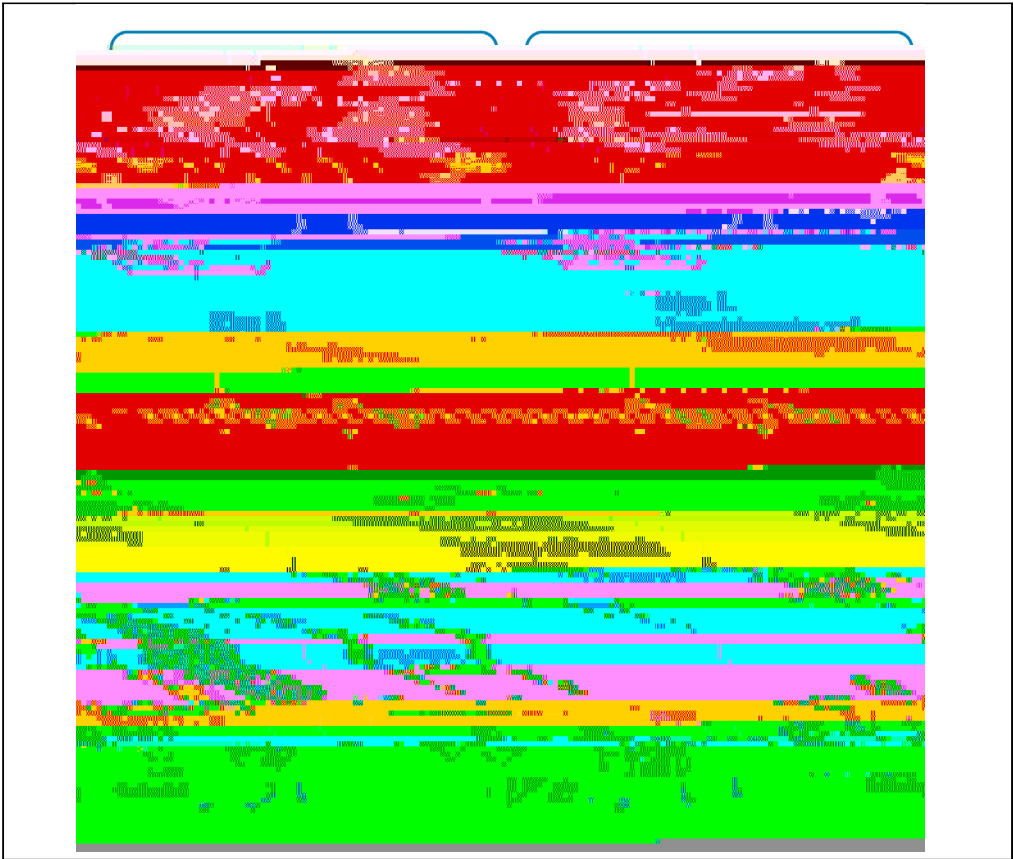


STAR Protocols

Optimized immunofluorescence staining protocol for imaging germinal centers in secondary lymphoid tissues of vaccinated mice



| fimm | f m | mi | | | | m |
 | | | | | f | mi | | | | | imi |
 imm | | | | | | | | | | | | | f |
 | f m | ● | | | | | | | | | | | if
 | | | | | | | | | | | | | |

| | F | | |
 im | | | |
 | | L | | |
 -V | |
 | m |
 | | F | | |
 m | (F |
 -V | | m |
 m | -V |
 | | |
 -mm | | | f |
 | f m |
 ● |
 imm | | mi |
 | | |
 imi |
 | f | f |
 im | | f | |
 mi | | |
	f	
		f
f		
F		
2 00		
m	. 0	© 0
		0 0
0 00		



Optimized immunofluorescence staining protocol for imaging germinal centers in secondary lymphoid tissues of vaccinated mice

Sigrid Fra-Bido,^{1,2,*} Simon A. Walker,¹ Silvia Innocentin,¹ and Michelle A. Linterman^{1,3,*}

¹The Babraham Institute, Babraham Research Campus, Cambridge, CB22 3AT, UK

²Technical contact

³Lead contact

*Correspondence: Sigrid.Fra-Bido@babraham.ac.uk (S.F.-B.), Michelle.Linterman@babraham.ac.uk (M.A.L.)
<https://doi.org/10.1016/j.xpro.2021.100499>

ABSTRACT

Location of immune cells that form the germinal center reaction within secondary lymphoid tissues can be characterized using confocal microscopy. Here, we present an optimized immunofluorescence staining protocol to image germinal center structures in fixed/frozen spleen sections from ChAdOx1 nCoV-19 immunized mice. This protocol can be adapted to identify other cell types within secondary lymphoid tissues.

For complete information on the generation and use of this protocol to examine immune responses to the COVID vaccine ChAdOx1 nCoV-19, please refer to Silva-Cayetano et al. (2020).

INTRODUCTION

Secondary lymphoid tissues are the sites where B cells undergo germinal center (GC) reactions, which are essential for the generation of high-affinity antibodies. The GC reaction is a complex process involving the proliferation and differentiation of B cells into memory B cells and plasma cells. The GC reaction is characterized by the formation of a dark-staining structure within the spleen, known as a germinal center. The GC reaction is a key component of the adaptive immune response and is essential for the generation of high-affinity antibodies. The GC reaction is a complex process involving the proliferation and differentiation of B cells into memory B cells and plasma cells. The GC reaction is characterized by the formation of a dark-staining structure within the spleen, known as a germinal center. The GC reaction is a key component of the adaptive immune response and is essential for the generation of high-affinity antibodies.

— mm i mi (m
i 0μ f × 0^g

m m i i i f m i m

Note: $\int \frac{1}{x} dx = \ln|x| + C$ if $x > 0$

L. ... + L.

△

Note: $m \quad | \quad -0^\circ \quad (\quad f \quad -M \quad m \quad -00 \quad -M \quad f \quad | \quad m \quad \circ \quad f \quad 00 \quad -M$

Note:

im i i i F
im i (.00. i i im
m i €

im _ (.00. E
(.00 J

f mi f im i i
((i i _

i i fF i ii

1
m j i

f i f i i m

f i i

... m i m i i 00 i i m i i
... m if i i f (J m . 0 0

U-I

$\frac{1}{R}$

F

$\frac{1}{R}$

$\frac{1}{R}$

m

f



Jonkman, J., Brown, C.M., Wright, G.D., Anderson, K.I., and North, A.J. (2020). Tutorial: guidance for quantitative confocal microscopy. *Nat. Protoc.* 15, 1585–1611.

McClean, I.W., and Nakane, P.K. (1974). Periodate-lysine-paraformaldehyde fixative a new fixative for immunoelectron microscopy. *J. Histochem. Cytochem.* 22, 1077–1083.

North, A.J. (2006). Seeing is believing? A beginners' guide to practical pitfalls in image