PerFix-nc, Workflow Protocols

PerFix-nc Proposed Research Protocols	PerFix-nc STRAIGHT	PerFix-nc WITH HI. FIX.	PerFix-nc + WASH	PerFix-nc WITH HI. FIX. + WASH	PerFix-nc FOR FRAGILE SURFACE EPITOPES	PerFix-nc FOR FoxP3	PerFix-nc FOR Zap-70
Specimen	Whole Blood	Whole Blood	Whole Blood	Whole Blood	Whole Blood	Whole Blood	Whole Blood
Surface Ab's Before?	NO	YES / NO**	NO	YES / NO**	YES	NO	NO
Intra Ab's: Alone? or Mixed with Surface?	Mixed	Alone / Mixed**	Mixed	Alone / Mixed**	Alone	Mixed	Mixed
Protocols	A	B	C	D	E	F	G
Surface Antibodies (Only if necessary)	NA	xx µL* / NA	NA	xx µL* / NA	xx µL*	NA	NA
Specimen Volume	50 µL	+ 50 µL	50 µL	+ 50 µL	+ 50 µL	50 µL	50 µL
Mixing step	NA	Vortex immediately	NA	Vortex immediately	Vortex immediately	NA	NA
Surface Ab Incubation	NA	15-20 min, RT (18-25°C), in the dark if surface Ab's	NA	15-20 min, RT (18-25°C), in the dark if surface Ab's	15-20 min, RT (18-25°C), in the dark	NA	NA
FIXATION REAGENT	PerFix-nc Buffer 1	PerFix-nc Buffer 1	PerFix-nc Buffer 1	PerFix-nc Buffer 1	PerFix-nc Buffer 1	PerFix-nc Buffer 1	PerFix-nc Buffer 1
Fixation volume	+ 5 µL	+ 25 µL	+ 5 µL	+ 25 µL	+ 5 µL or + 25 µL	+ 5 µL	+ 5 µL
Mixing step	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately
Fixative Incubation	15 min, RT (18-25°C)	15 min, RT (18-25°C) in the dark if surface Ab's	15 min, RT (18-25°C)	15 min, RT (18-25°C), in the dark if surface Ab's	15 min, RT (18-25°C), in the dark	15 min, RT (18-25°C)	15 min, RT (18-25°C)
Mixing step after incubation	Vortex again	Vortex again	Vortex again	Vortex again	Vortex again	Vortex again	Vortex again
PERMEABILIZATION REAGENT	PerFix-nc Buffer 2	PerFix-nc Buffer 2	PerFix-nc Buffer 2	PerFix-nc Buffer 2	PerFix-nc Buffer 2	PerFix-nc Buffer 2	PerFix-nc Buffer 2
Permeabilization volume	+ 300 µL	+ 300 µL	+ 300 µL	+ 300 µL	+ 300 µL	+ 300 µL	+ 300 µL
Mixed Surface & Intra Ab's	+ xx + yy µL*	+ xx + yy µL*	+ xx + yy µL*	+ xx + yy µL*	+ xx + yy µL*	+ xx + 10 µL anti-FoxP3 / clone 259D (Biolegend)	+ xx + yy µL*
Mixing step	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately
Perm + Staining Incubation	15-30 min, RT (18-25°C), in the dark	15-30 min, RT (18-25°C), in the dark	15-30 min, RT (18-25°C), in the dark	15-30 min, RT (18-25°C), in the dark	15-30 min, RT (18-25°C), in the dark	60 min, RT (18-25°C), in the dark	45 min, RT (18-25°C), in the dark

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INTERMEDIATE WASH REAGENT	NA	NA	NA	NA	NA	PBS	NA
Washing buffer volume	NA	NA	NA	NA	NA	+ 3,000 µL	NA
Mixing step	NA	NA	NA	NA	NA	Vortex immediately	NA
Intermediate Incubation	NA	NA	NA	NA	NA	5 min, RT (18-25°C), in the dark	NA
Intermediate Centrifugation	NA	NA	NA	NA	NA	Centrifuge 5 min, RT (18-25°C), @ 500 x g	NA
Decant supernatant?	NA	NA	NA	NA	NA	Yes	NA
FINALIZATION REAGENT	1/10 Diluted PerFix-nc Buffer 3	1/10 Diluted PerFix-nc Buffer 3	1/10 Diluted PerFix-nc Buffer 3	1/10 Diluted PerFix-nc Buffer 3	1/10 Diluted PerFix-nc Buffer 3	1/10 Diluted PerFix-nc Buffer 3	1/10 Diluted PerFix-nc Buffer 3
Final 1X Reagent volume	+ 3,000 µL	+ 3,000 µL	+ 3,000 µL	+ 3,000 µL	+ 3,000 µL	+ 3,000 µL	+ 3,000 µL
Mixing step	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately	Vortex immediately
FINAL CENTRIFUGATION	NA	NA	Centrifuge 5 min, RT (18-25°C), @ 500 x g	Centrifuge 5 min, RT (18-25°C), @ 500 x g	NA	CCentrifuge 5 min, RT (18-25°C), @ 500 x	Centrifuge 5 min, RT (18-25°C), @ 500 x g
Decant supernatant?	NA	NA	Yes	Yes	NA	Yes	Yes
Resuspension Buffer	NA	NA	1/10 Diluted PerFix-nc Buffer 3	1/10 Diluted PerFix-nc Buffer 3	NA	1/10 Diluted PerFix-nc Buffer 3	1/10 Diluted PerFix-nc Buffer 3
Final 1X Reagent volume	NA	NA	+ 500 µL	+ 500 µL	NA	+ 500 µL	+ 500 µL
Mixing step	NA	NA	Vortex immediately	Vortex immediately	NA	Vortex immediately	Vortex immediately
Analyze on a Flow Cytometer	Analyze within 2 h	Analyze within 2 h	Analyze within 24 h if stored at 2-8°C	Analyze within 24 h if stored at 2-8°C	Analyze within 2 h	Analyze within 24 h if stored at 2-8°C	Analyze within 24 h if stored at 2-8°C
BENEFITS / COMMENTS	Fast Preparation Simple Reproducible Accurate Automatable	Fast Preparation Simple Reproducible Accurate Automatable	Signal/Noise Improvement Faster Acquisition on FCM, Immunostaining stability	Signal/Noise Improvement Faster Acquisition on FCM Immunostaining stability	Any surface epitope can be included Wash is optional	FoxP3-optimized	Zap-70-optimized

With high fixative volume, emphasis is put on surface marker reactivity evaluation needs. It is recommended to titrate the antibody(ies) in parallel with the 2 procedures (normal and high fixation). Volumes of added Ab's (xx µL for Surface and yy µL for Intracellular Ab's) are to be defined precisely by careful titrations.

Tested products are labeled RUO (For Research use Only. Not for use in diagnostic procedures)

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