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 + yy µL*	+ 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
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	Centrifuge 5 min, RT (18-25°C), @ 500 x g	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.