
Supplementary information

IBEX: an iterative immunolabeling and chemical bleaching method for high-content imaging of diverse tissues

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Supplementary Information for

IBEX: An iterative immunolabeling and chemical bleaching method for high-content imaging of diverse tissues

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Tables S1-S4

Table S1. Time and method used to bleach fluorescently conjugated antibodies and dyes.

Antibody	Clone	RRID	Vendor	Cat No.	Species reactivity	Isotype	Dilution	Time to Bleach (Minutes)	
								LiBH ₄	LiBH ₄ + Light
β-3 Tubulin BV421	AA10	AB_2632699	BioLegend	657412	Human, Mouse	Mouse IgG2a, κ	1:200	-	<15
β-3 Tubulin BV421	TUJ1	AB_2564757	BioLegend	801203	Human, Mouse	Mouse IgG2a, κ	1:50	<15	-
β-3 Tubulin BV421	AA10	AB_2892767	BioLegend	Custom	Human, Mouse	Mouse IgG2a, κ	1:100	<15	-
ACE2	-	AB_355722	R&D	AF933	Human	Goat IgG	1:50	-	-
B220 BV421	RA3-6B2	AB_2562905	BioLegend	103251	Mouse	Rat IgG2a, κ	1:400	-	<15
B220 BV510	RA3-6B2	AB_2650679	BioLegend	103248	Mouse	Rat IgG2a, κ	1:300	-	<15
B220 AF488	RA3-6B2	AB_396781	BD Biosciences	557669	Mouse	Rat IgG2a, κ	1:400	<15	-
B220 AF532	RA3-6B2	AB_11218492	Thermo	58-0452-82	Mouse	Rat IgG2a, κ	1:50	<15	-
B220 PE	RA3-6B2	AB_394620	BD Biosciences	553090	Mouse	Rat IgG2a, κ	1:400	<15	-
B220 eF570	RA3-6B2	AB_2573598	Thermo	41-0452-80	Mouse	Rat IgG2a, κ	1:200	<15	-
B220 eF615	RA3-6B2	AB_10852702	Thermo	42-0452-82	Mouse	Rat IgG2a, κ	1:200	>120	-
B220 AF647	RA3-6B2	AB_389330	BioLegend	103226	Mouse	Rat IgG2a, κ	1:400	<15	-
B220 AF700	RA3-6B2	AB_493717	BioLegend	103232	Mouse	Rat IgG2a, κ	1:50	<15	-
BCL2 AF647	100	AB_2563279	BioLegend	658705	Human	Mouse IgG ₁	1:25	<15	-
Bcl6 AF647	K112-91	AB_10898007	BD Biosciences	561525	Human, Mouse	Mouse IgG ₁ , κ	1:25	<15	-
Cathepsin L	204101	AB_2087829	R&D	MAB-9521	Human	Rat IgG2a	1:50	-	-
CD1c PE	L161	AB_1089000	BioLegend	331506	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD1d PE	1B1	AB_2073521	BD Biosciences	553846	Mouse	Rat IgG2b, κ	1:100	<15	-
CD3 BV421	17A2	AB_2562553	BioLegend	100228	Mouse	Rat IgG2b, κ	1:400	-	<15
CD3 BV510	17A2	AB_2562555	BioLegend	100234	Mouse	Rat IgG2b, κ	1:50	-	<15
CD3 AF488	17A2	AB_389301	BioLegend	100210	Mouse	Rat IgG2b, κ	1:200	<15	-
CD3 AF532	17A2	AB_11220474	Thermo	58-0032-80	Mouse	Rat IgG2b, κ	1:50	<15	-
CD3 PE	17A2	AB_312662	BioLegend	100205	Mouse	Rat IgG2b, κ	1:200	<15	-
CD3 AF594	17A2	AB_2563427	BioLegend	100240	Mouse	Rat IgG2b, κ	1:400	>120	-
CD3 AF647	17A2	AB_396912	BD Biosciences	557869	Mouse	Rat IgG2b, κ	1:400	<15	-
CD3 AF532	UCHT1	AB_11218675	Thermo	58-0038-42	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD3 AF594	UCHT1	AB_2563236	BioLegend	300446	Human	Mouse IgG ₁	1:200	>120	-
CD3 iF594	UCHT1	AB_2892737	Caprico Biotechnologies	1053134	Human	Mouse IgG ₁	1:50	<15	-
CD4 BV421	GK1.5	AB_2562557	BioLegend	100443	Mouse	Rat IgG2b, κ	1:200	-	<15
CD4 BV510	GK1.5	AB_2564587	BioLegend	100449	Mouse	Rat IgG2b, κ	1:50	-	<15
CD4 PE	GK1.5	AB_395014	BD Biosciences	553730	Mouse	Rat IgG2b, κ	1:200	<15	-
CD4 AF594	GK1.5	AB_2563182	BioLegend	100446	Mouse	Rat IgG2b, κ	1:200	>120	-
CD4 AF488	RM4-5	AB_396779	BD Biosciences	557667	Mouse	Rat IgG2a, κ	1:100	<15	-
CD4 AF532	RM4-5	AB_11219484	Thermo	58-0042-80	Mouse	Rat IgG2a, κ	1:50	<15	-
CD4 eF570	RM4-5	AB_2573595	Thermo	41-0042-82	Mouse	Rat IgG2a, κ	1:100	<15	-
CD4 AF532	RPA-T4	AB_2802361	Thermo	58-0049-42	Human	Mouse IgG ₁ , κ	1:25	<15	-
CD4 AF647	RPA-T4	AB_389333	BioLegend	300520	Human	Mouse IgG ₁ , κ	1:100	<15	-

CD4 AF700	RPA-T4	AB_493743	BioLegend	300526	Human	Mouse IgG ₁ , κ	1:25	<15	-
CD8 BV421	53-6.7	AB_11204079	BioLegend	100738	Mouse	Rat IgG2a, κ	1:200	-	<15
CD8 BV510	53-6.7	AB_2563057	BioLegend	100752	Mouse	Rat IgG2a, κ	1:200	-	<15
CD8 AF488	53-6.7	AB_389304	BioLegend	100723	Mouse	Rat IgG2a, κ	1:200	<15	-
CD8 PE	53-6.7	AB_394570	BD Biosciences	553032	Mouse	Rat IgG2a, κ	1:400	<15	-
CD8 AF594	53-6.7	AB_2563237	BioLegend	100758	Mouse	Rat IgG2a, κ	1:200	>120	-
CD8 AF647	53-6.7	AB_389326	BioLegend	100724	Mouse	Rat IgG2a, κ	1:200	<15	-
CD8 AF488	SK1	AB_10549301	BioLegend	344716	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD10 PE	FR4D11	NA	Caprico Biotechnologies	103926	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD10	-	AB_354652	R&D	AF1182	Human	Goat IgG	1:200	-	-
CD11b FITC	5C6	AB_2538034	Thermo	MA5-16529	Mouse	Rat IgG2b	1:100	<30	-
CD11b PE	5C6	AB_323678	Bio-Rad	MCA711PE	Mouse	Rat IgG2b	1:100	<15	-
CD11b AF488	M1/70	AB_389305	BioLegend	101217	Mouse	Rat IgG2b, κ	1:100	<15	-
CD11c AF488	N418	AB_10373244	Thermo	MCD11c20	Mouse	Hamster IgG	1:50	<15	-
CD11c AF594	N418	AB_2563323	BioLegend	117346	Mouse	Hamster IgG	1:50	>120	-
CD11c AF647	N418	AB_389328	BioLegend	117312	Mouse	Hamster IgG	1:100	<15	-
CD11c AF700	B-Ly6	AB_10612006	BD Biosciences	561352	Human	Mouse IgG ₁ , κ	1:25	<15	-
CD15	MMA	AB_400298	BD Biosciences	347420	Human	Mouse IgM	1:50	-	-
CD20 AF488	L26	AB_10734358	Thermo	53-0202-82	Human	Mouse IgG2b, κ	1:200	<15	-
CD20 eF615	L26	AB_10853517	Thermo	42-0202-82	Human	Mouse IgG2b, κ	1:200	>120	-
CD20 eF660	L26	AB_11150959	Thermo	50-0202-82	Human	Mouse IgG2b, κ	1:200	<15	-
CD21 Pacific Blue	7E9	AB_2085159	BioLegend	123413	Mouse	Rat IgG2a, κ	1:200	<15	-
CD21 AF488	Bu32	AB_2892738	BioLegend	Custom	Human	Mouse IgG ₁ , κ	1:100	<15	-
CD21 AF532	Bu32	AB_2892739	BioLegend	Custom	Human	Mouse IgG ₁ , κ	1:400	<15	-
CD23 AF647	B3B4	AB_493479	BioLegend	101611	Mouse	Rat IgG2a, κ	1:50	<15	-
CD23 AF532	EBVCS-5	AB_2892740	BioLegend	Custom	Human	Mouse IgG ₁ , κ	1:25	<15	-
CD25 AF488	PC61.5	AB_763472	Thermo	53-0251-82	Mouse	Rat IgG1	1:50	<15	-
CD25 AF647	M-A251	AB_2563587	BioLegend	356127	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD31 AF488	MEC13.3	AB_2161031	BioLegend	102514	Mouse	Rat IgG2a, κ	1:100	<15	-
CD31 PE	MEC13.3	AB_394819	BD Biosciences	553373	Mouse	Rat IgG2a, κ	1:200	<15	-
CD31 AF594	MEC13.3	AB_2563319	BioLegend	102520	Mouse	Rat IgG2a, κ	1:100	>120	-
CD31 AF647	MEC13.3	AB_2161029	BioLegend	102516	Mouse	Rat IgG2a, κ	1:100	<15	-
CD31 PE	WM59	AB_314332	BioLegend	303106	Human	Mouse IgG ₁ , κ	1:100	<15	-
CD31 AF700	WM59	AB_2566326	BioLegend	303133	Human	Mouse IgG ₁ , κ	1:25	<15	-
CD34 PE	QBEND/10	AB_11152571	Thermo	MA1-10205	Human	Mouse IgG ₁	1:50	<15	-
CD34 iF594	4H11	AB_2892741	AAT Bioquest	103400C0	Human	Mouse IgG ₁	1:25	<15	-
CD35 BV510	8C12	AB_2739889	BD Biosciences	740132	Mouse	Rat IgG2a, κ	1:600	-	<15
CD35 PE	E11	AB_2292231	BioLegend	333406	Human	Mouse IgG ₁ , κ	1:800	<15	-
CD38 AF700	HIT2	AB_2072781	BioLegend	303524	Human	Mouse IgG ₁ , κ	1:25	<15	-
CD39 PE	A1	AB_940429	BioLegend	328208	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD44 AF488	IM7	AB_493679	BioLegend	103016	Human, Mouse	Rat IgG2b, κ	1:100	<15	-

CD44 AF532	IM7	AB_2892768	BioLegend	Custom	Human, Mouse	Rat IgG2b, κ	1:100	<15	-
CD44 AF647	IM7	AB_493681	BioLegend	103018	Human, Mouse	Rat IgG2b, κ	1:100	<15	-
CD44 AF700	IM7	AB_493713	BioLegend	103026	Human, Mouse	Rat IgG2b, κ	1:50	<15	-
CD45 BV421	30-F11	AB_2562559	BioLegend	103134	Mouse	Rat IgG2b, κ	1:100	-	<15
CD45 BV510	30-F11	AB_2563061	BioLegend	103138	Mouse	Rat IgG2b, κ	1:100	-	<15
CD45 AF488	30-F11	AB_493531	BioLegend	103122	Mouse	Rat IgG2b, κ	1:200	<15	-
CD45 AF532	30-F11	AB_11218871	Thermo	58-0451-82	Mouse	Rat IgG2b, κ	1:200	<15	-
CD45 AF647	30-F11	AB_493533	BioLegend	103124	Mouse	Rat IgG2b, κ	1:400	<15	-
CD45 AF700	30-F11	AB_493715	BioLegend	103128	Mouse	Rat IgG2b, κ	1:50	<15	-
CD45 AF532	HI30	AB_11218084	Thermo	58-0459-41	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD45 AF594	HI30	AB_2629599	BioLegend	304060	Human	Mouse IgG ₁ , κ	1:100	>120	-
CD45 PE/iFluor594	F10-89-4	AB_2892742	Caprico Biotechnologies	1016185	Human	Mouse IgG2a, κ	1:50	<15	-
CD45 iF594	F10-89-4	AB_2892743	Caprico Biotechnologies	1016136	Human	Mouse IgG2a, κ	1:50	<15	-
CD49a PE	TS2/7	AB_1236441	BioLegend	328304	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD49a AF647	TS2/7	AB_2129242	BioLegend	328310	Human	Mouse IgG ₁ , κ	1:200	<15	-
CD54 AF647	HA58	AB_2715942	BioLegend	353114	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD61 FITC	Y2/51	AB_2660493	Miltenyi	130-098-682	Human	Mouse IgG ₁ , κ	1:25	<30	-
CD64 AF647	X54-5/7.1	AB_2566561	BioLegend	139322	Mouse	Mouse IgG ₁ , κ	1:50	<15	-
CD66b iF594	G10F5	AB_2892744	Caprico Biotechnologies	1075139	Human	Mouse IgM, κ	1:25	<15	-
CD66b AF647	G10F5	AB_2563171	BioLegend	305110	Human	Mouse IgM, κ	1:25	<15	-
CD68 BV421	FA-11	AB_2562949	BioLegend	137017	Mouse	Rat IgG2a	1:200	-	<15
CD68 AF488	FA-11	AB_2074847	BioLegend	137011	Mouse	Rat IgG2a	1:50	<15	-
CD68 iF594	KP1	AB_2892745	Caprico Biotechnologies	1064135	Human	Mouse IgG ₁ , κ	1:100	<15	-
CD68 AF647	KP1	AB_627158	Santa Cruz	sc-200060	Human	Mouse IgG ₁ , κ	1:100	<15	-
CD69	-	AB_416586	R&D	AF2386	Mouse	Goat IgG	1:50	-	-
CD69 AF647	FN50	AB_528871	BioLegend	310918	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD94 PE	DX22	AB_314536	BioLegend	305506	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD103 AF488	2E7	AB_535949	BioLegend	121407	Mouse	Hamster IgG	1:100	<15	-
CD106 AF488	429	AB_493427	BioLegend	105710	Mouse	Rat IgG2a, κ	1:50	<15	-
CD106 eF660	429	AB_11217676	Thermo	50-1061-80	Mouse	Rat IgG2a, κ	1:50	<15	-
CD106 PE	STA	AB_314561	BioLegend	305806	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD117 BV421	2B8	AB_10898120	BioLegend	105827	Mouse	Rat IgG2b, κ	1:50	-	<15
CD117 AF488	104D2	AB_2566221	BioLegend	313234	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD117 PE	104D2	AB_314983	BioLegend	313204	Human	Mouse IgG ₁ , κ	1:50	<15	-
CD138 BV421	281-2	AB_11153126	BD Biosciences	562610	Mouse	Rat IgG2a, κ	1:50	-	<15
CD138 AF647	281-2	AB_2566239	BioLegend	142526	Mouse	Rat IgG2a, κ	1:50	<15	-
CD138 PE	MI15	AB_2561878	BioLegend	356504	Human	Mouse IgG ₁ , κ	1:200	<15	-
CD138 AF647	MI15	AB_2564250	BioLegend	356523	Human	Mouse IgG ₁ , κ	1:200	<15	-
CD163 PE	GH1/61	AB_1134002	BioLegend	333606	Human	Mouse IgG ₁ , κ	1:300	<15	-
CD163 AF647	GH1/61	AB_2563474	BioLegend	333620	Human	Mouse IgG ₁ , κ	1:100	<15	-
CD166 AF488	EPR2759	AB_2889210	Abcam	ab197543	Human	Rabbit mAb	1:100	<15	-

CD169 FITC	3D6.112	AB_324246	Bio-Rad	MCA884F	Mouse	Rat IgG2a, κ	1:50	<30	-
CD169 PE	3D6.112	AB_10915697	BioLegend	142404	Mouse	Rat IgG2a, κ	1:300	<15	-
CD169 AF594	3D6.112	AB_2565620	BioLegend	142416	Mouse	Rat IgG2a, κ	1:100	>120	-
CD200 BV421	Ox-90	AB_2739289	BD Biosciences	565547	Mouse	Rat IgG2a, κ	1:100	-	<15
CD206 BV421	C068C2	AB_2562232	BioLegend	141717	Mouse	Rat IgG2a, κ	1:100	-	<15
CD207 PE	eBioL31	AB_763453	Thermo	12-2075-80	Mouse	Rat IgG2a	1:50	<15	-
Clec9a AF488	8F9	NA	BioLegend	Custom	Human	Mouse IgG2a, κ	1:25	<15	-
Collagen IV	-	AB_445160	Abcam	ab19808	Mouse	Rabbit IgG	1:200	-	-
Collagen IV	-	AB_305584	Abcam	ab6586	Human	Rabbit IgG	1:200	-	-
CXCL12 AF532	79018	-	R&D	MAB350-500 (Unconjugated)	Human	Mouse IgG ₁	1:25	<15	-
CXCL13 AF532	-	AB_355613	R&D	AF801 (Unconjugated)	Human	Goat IgG	1:25	<15	-
CXCR6 AF647	221002	NA	Novus	FAB2145R	Mouse	Rat IgG2b	1:50	<15	-
Cytokeratin AF647	C-11	AB_2563652	BioLegend	628604	Human	Mouse IgG ₁ , κ	1:200	<15	-
Cytokeratin AF488	AE1/AE3	AB_2574301	Thermo	53-9003-82	Human, Mouse	Mouse IgG ₁	1:50	<15	-
Cytokeratin eF660	AE1/AE3	AB_2574301	Thermo	50-9003-82	Human, Mouse	Mouse IgG ₁	1:100	<15	-
Cytokeratin 7 AF488	W16155A	AB_2728458	BioLegend	601605	Human	Rat IgG2a, κ	1:50	<15	-
Cytokeratin 10 AF488	DE-K10	AB_2892746	Novus	NBP2-54402AF488	Human	Mouse IgG ₁ , κ	1:200	<15	-
Cytokeratin 14	Poly9060	AB_2616962	BioLegend	906004	Human, Mouse	Chicken IgY	1:50	-	-
DCAMKL1	-	AB_873538	Abcam	ab37994	Human, Mouse	Rabbit IgG	1:50	-	-
DC-SIGN AF647	9E9A8	AB_1186092	BioLegend	330112	Human	Mouse IgG2a, κ	1:50	<15	-
DEC205 AF647	NLDC-145	AB_2137655	BioLegend	138204	Mouse	Rat IgG2a, κ	1:50	<15	-
Desmin	-	AB_301744	Abcam	ab15200	Human	Rabbit IgG	1:200	-	-
Desmin AF488	Y66	AB_2892748	Abcam	ab185033	Human	Rabbit IgG	1:200	<15	-
E-cadherin AF647	DECMA-1	AB_2563955	BioLegend	147308	Mouse	Rat IgG1, κ	1:100	<15	-
EpCAM BV510	G8.8	AB_2738075	BD Biosciences	563216	Mouse	Rat IgG2a, κ	1:100	-	<15
EpCAM AF594	G8.8	AB_2563322	BioLegend	118222	Mouse	Rat IgG2a, κ	1:400	>120	-
EpCAM AF647	G8.8	AB_1134101	BioLegend	118212	Mouse	Rat IgG2a, κ	1:200	<15	-
EpCAM AF488	9C4	AB_756084	BioLegend	324210	Human	Mouse IgG2b, κ	1:300	<15	-
EpCAM AF594	9C4	AB_2563209	BioLegend	324228	Human	Mouse IgG2b, κ	1:500	>120	-
EpCAM AF647	9C4	AB_1134101	BioLegend	324212	Human	Mouse IgG2b, κ	1:100	<15	-
EpCAM	-	AB_1603782	Abcam	ab71916	Human	Rabbit IgG	1:50	-	-
F4/80 BV421	BM8	AB_11203717	BioLegend	123132	Mouse	Rat IgG2a, κ	1:50	-	<15
F4/80 PE	BM8	AB_465923	Thermo	12-4801-82	Mouse	Rat IgG2a, κ	1:100	<15	-
F4/80 AF647	BM8	AB_893480	BioLegend	123122	Mouse	Rat IgG2a, κ	1:50	<15	-
F4/80 AF700	BM8	AB_2293450	BioLegend	123130	Mouse	Rat IgG2a, κ	1:50	<15	-
Fibronectin AF532	2F4	NA	Novus	NBP2-22113AF532	Human	Mouse IgG ₁	1:25	<15	-
Fibrinogen	-	AB_10900171	Abcam	ab118533 (Unconjugated)	Human	Sheep IgG	1:200	<15	-
Foxp3 AF488	FJK-16s	AB_763537	Thermo	53-5773-82	Mouse	Rat IgG2a, κ	1:50	<15	-
Foxp3 AF532	FJK-16s	AB_11218870	Thermo	58-5773-82	Mouse	Rat IgG2a, κ	1:50	<15	-
Foxp3 PE	FJK-16s	AB_465936	Thermo	12-5773-82	Mouse	Rat IgG2a, κ	1:50	<15	-
Foxp3 eF570	FJK-16s	AB_11219073	Thermo	41-5773-82	Mouse	Rat IgG2a, κ	1:50	<15	-

Foxp3 eF660	FJK-16s	AB_11218868	Thermo	50-5773-82	Mouse	Rat IgG2a, κ	1:50	<15	-
FOXP3 eF570	236A/E7	AB_2573609	Thermo	41-4777-82	Human	Mouse IgG ₁ , κ	1:25	<15	-
GL-7 PE	GL7	AB_10715834	BD Biosciences	561530	Mouse	Rat IgM, κ	1:100	<15	-
Glutamine synthetase	-	AB_880241	Abcam	ab49873	Human, Mouse	Rabbit IgG	1:200	-	-
Glycophorin	EPR8200	AB_2892758	Abcam	ab218372	Human	Rabbit IgG	1:300	-	-
gp38 AF488	8.1.1	AB_1133992	BioLegend	127405	Mouse	Hamster IgG	1:50	<15	-
HLA-DR AF488	L243	AB_493176	BioLegend	307619	Human	Mouse IgG2a, κ	1:200	<15	-
HLA-DR iF594	L243	AB_2892750	Caprico Biotechnologies	1032136	Human	Mouse IgG2a, κ	1:100	<15	-
Hoechst 33342	-	-	Biotium	40046	-	-	1:5,000	-	-
ICOS AF488	C398.4A	AB_2122584	BioLegend	313514	Human	Hamster IgG	1:25	<15	-
IgA AF555	-	AB_2794378	SouthernBiotech	1040-32	Human	Goat IgG	1:500	<15	-
IgA1 AF647	B3506B4	AB_2796658	SouthernBiotech	9130-31	Human	Mouse IgG ₁ , κ	1:500	<15	-
IgA2 AF488	A9604D2	AB_2796665	SouthernBiotech	9140-30	Human	Mouse IgG ₁ , κ	1:500	<15	-
IgD AF488	11-26c.2a	AB_10730619	BioLegend	405718	Mouse	Rat IgG2a, κ	1:400	<15	-
IgD AF594	11-26c.2a	AB_2565572	BioLegend	405740	Mouse	Rat IgG2a, κ	1:400	>120	-
IgD AF700	11-26c.2a	AB_2563340	BioLegend	405729	Mouse	Rat IgG2a, κ	1:50	<15	-
IgD AF488	IA6-2	AB_11150397	BioLegend	348216	Human	Mouse IgG2a, κ	1:25	<15	-
IgD AF647	IA6-2	AB_2563269	BioLegend	348228	Human	Mouse IgG2a, κ	1:100	<15	-
IgM AF647	EPR5539-65-4	NA	Abcam	ab200629	Human	Rabbit mAb	1:100	<15	-
IRF4 FITC	3E4	AB_2572538	Thermo	11-9858-82	Human, Mouse	Rat IgG1, κ	1:50	<30	-
IRF4 PE	3E4	AB_2563004	Thermo	12-9858-82	Human, Mouse	Rat IgG1, κ	1:50	<15	-
JOJO-1	-	-	Thermo	J11372	-	-	1:10,000	-	-
Ki-67 AF488	B56	AB_647087	BD Biosciences	558616	Human, Mouse	Mouse IgG ₁ , κ	1:50	<15	-
Ki-67 AF700	B56	AB_10611571	BD Biosciences	561277	Human, Mouse	Mouse IgG ₁ , κ	1:50	<15	-
KLRG1 AF488	2F1	AB_10898017	BD Biosciences	561619	Mouse	Hamster IgG ₂ , κ	1:50	<15	-
Laminin 1 + 2	-	AB_305933	Abcam	ab7463	Human, Mouse	Rabbit IgG	1:100	-	-
Langerin	929F3.01	AB_2892751	Novus	DDX0362P-100	Human	Rat IgG2a	1:50	-	-
Lumican AF532	-	AB_2139484	R&D	AF2846 (Unconjugated)	Human	Goat IgG	1:50	<15	-
Lumican AF700	-	AB_2139484	R&D	AF2846 (Unconjugated)	-	Goat IgG	1:25	<15	-
Ly-6G AF488	1A8	AB_2561340	BioLegend	127626	Mouse	Rat IgG2a, κ	1:50	<15	-
Lysozyme	-	AB_303050	Abcam	Ab2408	Human	Rabbit IgG	1:50	-	-
Lyve-1 eF570	ALY7	AB_2573596	Thermo	41-0443-82	Mouse	Rat IgG1, κ	1:100	<15	-
Lyve-1	-	AB_355144	R&D	AF2089 (Unconjugated)	Human	Goat IgG	1:100	<15	-
MARCO	-	AB_2643767	Thermo	PA5-64134	Human	Rabbit IgG	1:25	-	-
MHC-II BV421	M5/114.15.2	AB_10900075	BioLegend	107631	Mouse	Rat IgG2b, κ	1:400	-	<15
MHC-II AF647	M5/114.15.2	AB_493525	BioLegend	107618	Mouse	Rat IgG2b, κ	1:600	<15	-
MHC-II AF700	M5/114.15.2	AB_493727	BioLegend	107622	Mouse	Rat IgG2b, κ	1:100	<15	-
NF-H/NF-M AF488	SM1-35	AB_2750328	BioLegend	835614	Human	Mouse IgG ₁ , κ	1:50	<15	-
NK1.1 BV421	PK136	AB_10895916	BioLegend	108731	Mouse	Mouse IgG2a, κ	1:50	-	<15
p53 PE	PAb 240	NA	Novus	NB200-103PE	Human	Mouse IgG ₁ , κ	1:50	<15	-
Pax5 AF647	1H9	AB_2562425	BioLegend	649704	Mouse	Rat IgG2a, κ	1:100	<15	-

PD-1 BV421	29F.1A.12	AB_10900085	BioLegend	135217	Mouse	Rat IgG2a, κ	1:100	-	<15
PD-1 PE	29F.1A.12	AB_1877231	BioLegend	135206	Mouse	Rat IgG2a, κ	1:100	<15	-
PD-1 AF488	EH12.2H7	AB_2563594	BioLegend	329936	Human	Mouse IgG ₁ , κ	1:200	<15	-
PD-1 PE	EH12.2H7	AB_940481	BioLegend	329906	Human	Mouse IgG ₁ , κ	1:200	<15	-
PD-1 AF647	EH12.2H7	AB_940471	BioLegend	329910	Human	Mouse IgG ₁ , κ	1:200	<15	-
Podocin	JB51-33	AB_2892752	Novus	NBP2-75624	Human	Rabbit IgG	1:50	-	-
RORyt	AFKJS-9	AB_1834475	Thermo	14-6988-82	Mouse	Rat IgG2a	1:200	-	-
SiglecF PE	E50-2440	AB_394341	BD Biosciences	552126	Mouse	Rat IgG2a, κ	1:100	<15	-
SiglecF AF700	1RNM44N	AB_2637126	Thermo	56-1702-80	Mouse	Rat IgG2a, κ	1:50	<15	-
SIRPα AF488	P84	AB_2650815	BioLegend	144024	Mouse	Rat IgG1, κ	1:50	<15	-
SIRPα AF647	P84	AB_2721300	BioLegend	144027	Mouse	Rat IgG1, κ	1:200	<15	-
αSMA AF488	1A4	AB_2574460	Thermo	53-9760-80	Human, Mouse	Mouse IgG2a, κ	1:500	<15	-
αSMA eF570	1A4	AB_2573631	Thermo	41-9760-82	Human, Mouse	Mouse IgG2a, κ	1:300	<15	-
αSMA eF660	1A4	AB_2574461	Thermo	53-9760-82	Human, Mouse	Mouse IgG2a, κ	1:500	<15	-
SPARC AF532	-	AB_355728	R&D	AF941 (Unconjugated)	Human	Goat IgG	1:50	<15	-
Syndecan-1	-	AB_442186	R&D	AF2780	Human	Goat IgG	1:50	-	-
TCRγδ AF488	GL3	AB_2562771	BioLegend	118128	Mouse	Hamster IgG	1:50	<15	-
TCRγδ PE	GL3	AB_313832	BioLegend	118108	Mouse	Hamster IgG	1:100	<15	-
TCRγδ PE	B1	AB_1089218	BioLegend	331210	Human	Mouse IgG ₁ , κ	1:100	<15	-
Tim-3 AF532	344823	AB_2232900	R&D	MAB2365 (Unconjugated)	Human	Rat IgG2a	1:25	<15	-
Tim-3 AF647	344823	AB_2232900	R&D	MAB2365 (Unconjugated)	Human	Rat IgG2a	1:50	<15	-
Tim-4 BV421	21H12	AB_2741037	BD Biosciences	742773	Mouse	Rat IgG1, κ	1:100	-	<15
TMPRSS2	EPR3861	AB_10585592	Abcam	Ab92323	Human	Rabbit IgG	1:50	-	-
Tryptase	AA1	AB_303023	Abcam	Ab2378	Human	Mouse IgG ₁	1:50	-	-
Uromodulin FITC	10.32	AB_10012622	Novus	NBP1-50431	Human	Mouse IgG2b	1:25	<30	-
TCR Va7.2 AF647	3C10	AB_2566335	BioLegend	351726	Human	Mouse IgG ₁ , κ	1:50	<15	-
Vimentin AF532	O91D3	AB_2892753	BioLegend	Custom	Human	Mouse IgG2a	1:200	<15	-
Vimentin AF594	O91D3	AB_2566179	BioLegend	677804	Human	Mouse IgG2a	1:300	>120	-
Vimentin AF647	O91D3	AB_2616801	BioLegend	677807	Human	Mouse IgG2a	1:600	<15	-
anti-chicken IgY FITC	-	AB_923386	Thermo	SA1-72000	-	Donkey IgG	1:200	<30	-
anti-chicken IgY AF555	-	AB_2762844	Thermo	A32932	-	Goat IgG	1:400	<15	-
anti-goat IgG AF488	-	AB_2534102	Thermo	A-11055	-	Donkey IgG	1:400	<15	-
anti-goat IgG AF555	-	AB_2535853	Thermo	A21432	-	Donkey IgG	1:300	<15	-
anti-goat IgG AF680	-	AB_2535741	Thermo	A21084	-	Donkey IgG	1:300	<15	-
anti-hamster IgG AF647	-	AB_2535868	Thermo	A-21451	-	Goat IgG	1:400	<15	-
anti-mouse IgM AF488	-	AB_2340844	Jackson Immunoresearch	715-545-020	-	Donkey IgG	1:200	<15	-
anti-rabbit IgG AF488	-	AB_2576217	Thermo	A-11034	-	Goat IgG	1:400	<15	-
anti-rabbit IgG AF532	-	AB_2534076	Thermo	A-11009	-	Goat IgG	1:400	<15	-
anti-rabbit IgG AF555	-	AB_141784	Thermo	A-21428	-	Goat IgG	1:400	<15	-
anti-rabbit IgG AF594	-	AB_2534095	Thermo	A-11037	-	Goat IgG	1:400	>120	-
anti-rabbit IgG AF647	-	AB_2535813	Thermo	A-21245	-	Goat IgG	1:400	<15	-

anti-rabbit IgG AF700	-	AB_2535709	Thermo	A-21038	-	Goat IgG	1:400	<15	-
anti-rabbit IgG AF750	-	AB_2535710	Thermo	A-21039	-	Goat IgG	1:400	<15	-
anti-rabbit IgG AF488	-	AB_2535792	Thermo	A-21206	-	Donkey IgG	1:300	<15	-
anti-rabbit IgG AF555	-	AB_162543	Thermo	A-31572	-	Donkey IgG	1:300	<15	-
anti-rabbit IgG AF647	-	AB_2536183	Thermo	A-31573	-	Donkey IgG	1:400	<15	-
anti-rabbit IgG AF488	-	AB_2572214	Thermo	Z-25302	-	Goat Fab ₂	-	<15	-
anti-rabbit IgG AF532	-	AB_2736945	Thermo	Z-25303	-	Goat Fab ₂	-	<15	-
anti-rabbit IgG AF555	-	AB_2736950	Thermo	Z-25305	-	Goat Fab ₂	-	<15	-
anti-rabbit IgG AF594	-	AB_2736956	Thermo	Z-25307	-	Goat Fab ₂	-	>120	-
anti-rabbit IgG AF647	-	AB_2736962	Thermo	Z-25308	-	Goat Fab ₂	-	<15	-
anti-rat IgG AF647	-	AB_141778	Thermo	A-21247	-	Goat IgG	1:400	<15	-
anti-rat IgG AF647	-	AB_2340694	Jackson Immunoresearch	712-605-153	-	Donkey IgG	1:200	<15	-
anti-sheep IgG AF680	-	AB_2535755	Thermo	A-21102	-	Donkey IgG	1:300	<15	-

Table S2. IBEX panels for human organs.

Lymph node (Manual IBEX): 38 parameters, 9 cycles

Cycle	Marker	Clone	Conjugate	Isotype	Vendor	Catalog Number	Dilution
1	Hoechst	-	-		Biotium	40046	1:5000
	CD20	L26	AF488	Mouse IgG2b, κ	eBioscience	53-0202-82	1:100
	CD10	FR4D11	PE	Mouse IgG1, κ	Caprico Biotechnologies	103924	1:25
	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:50
	BCL2	100	AF647	Mouse IgG1	BioLegend	658705	1:25
	Collagen IV	Polyclonal rabbit IgG	Goat anti-Rabbit IgG AF700	Rabbit IgG Goat IgG	Abcam Thermo	Ab6586 A-21038	1:50 1:250
2	IgD	IA6-2	AF488	Mouse IgG2a, κ	BioLegend	348215	1:25
	CD23	EBVCS-5	AF532	Mouse IgG1, κ	BioLegend	Custom	1:25
	CD138	MI15	PE	Mouse IgG1, κ	BioLegend	356503	1:200
	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:100
	BCL6	K112-91	AF647	Mouse IgG1, κ	BD Biosciences	561525	1:10
	CD31	WM59	AF700	Mouse IgG1, κ	BioLegend	303133	1:25
3	HLA-DR	L243	AF488	Mouse IgG2a, κ	BioLegend	307619	1:100
	CD21	Bu32	AF532	Mouse IgG1, κ	BioLegend	NA, Custom	1:600
	CD1c	L161	PE	Mouse IgG1, κ	BioLegend	331505	1:50
	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:100
	CD163	GH1/61	AF647	Mouse IgG1, κ	BioLegend	333620	1:100
	CD11c	B-Ly6	AF700	Mouse IgG1, κ	BD Biosciences	561352	1:25
4	CD8	SK1	AF488	Mouse IgG1, κ	BioLegend	344716	1:50
	FOXP3	236A/E7	eF570	Mouse IgG1, κ	eBioscience	41-4777-82	1:50
	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:50
	CD25	M-A251	AF647	Mouse IgG1, κ	BioLegend	356127	1:50
	CD4	RPA-T4	FITC	Mouse IgG1, κ	Thermo	58-0049-41	1:25
	SPARC	Goat IgG	AF532	Goat IgG	R&D	AF941	1:25
5	PD-1	EH12.2H7	PE	Mouse IgG1, κ	BioLegend	329905	1:200
	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:50
	CD69	FN50	AF647	Mouse IgG1, κ	BioLegend	310918	1:25
	CD38	HIT2	AF700	Mouse IgG1, κ	BioLegend	303524	1:25
	ICOS	C398.4A	AF488	Hamster IgG	BioLegend	313514	1:25
	CXCL13	Goat IgG	AF532	Goat IgG	R&D	AF801 (Unconjugated)	1:25
6	IRF4	IRF4.3E4	PE	Rat IgG1, κ	BioLegend	646403	1:50
	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:50
	CD68	KP1	AF647	Mouse IgG1, κ	Santa Cruz	sc-20060	1:100
	Ki-67	B56	AF700	Mouse IgG1, κ	BD Biosciences	561277	1:50
	CD44	IM7	AF532	Rat IgG2b, κ	BioLegend	Custom	1:50
	CD35	E11	PE	Mouse IgG1, κ	BioLegend	333406	1:800
7	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:100
	Vα7.2	3C10	AF647	Mouse IgG1, κ	BioLegend	351726	1:50
	SMA-alpha	1A4	AF488	Mouse IgG2a, κ	Thermo	53-9760-80	1:100
	CD106	STA	PE	Mouse IgG1, κ	BioLegend	305805	1:50
	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:50
	Clec9a	8F9	AF488	Mouse IgG2a, κ	BioLegend	Custom	1:25
9	Lyve-1	Polyclonal goat IgG	AF532	Goat IgG	R&D	AF2089 (Unconjugated)	1:100
	CD45	F10-89-4	PE-iF594	Mouse IgG2a, κ	Caprico Biotechnologies	1016185	1:100
	CD3	UCHT1	AF594	Mouse IgG1, κ	BioLegend	300446	1:50
	DC-SIGN	9E9A8	AF647	Mouse IgG2a, κ	BioLegend	330112	1:50

Spleen (Manual IBEX): 25 parameters, 4 cycles

Cycle	Marker	Clone	Conjugate	Isotype	Vendor	Catalog Number	Dilution
1	Hoechst	-	-		Biotium	40046	1:5000
	CD20	L26	AF488	Mouse IgG2b, κ	eBioscience	53-0202-82	1:200
	CD21	Bu32	AF532	Mouse IgG1, κ	BioLegend	NA, Custom	1:400
	Glycophorin AF555	EPR8200	AF555	Rabbit IgG	Abcam Abcam	Ab218372 Ab269820	1:300
	CD68	KP1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1064135	1:50
	CD54	HA58	AF647	Mouse IgG1, κ	BioLegend	353114	1:100
	Fibrinogen	Polyclonal sheep IgG Polyclonal donkey IgG	Donkey anti-sheep IgG AF680	Sheep IgG Donkey IgG	Abcam Thermo	Ab118533 A-21102	1:200 1:400
	CD15	MMA Donkey anti-mouse IgM	Donkey anti-mouse IgM AF488	Mouse IgM, κ Donkey IgG	BD Biosciences Jackson Immunoresearch	347420 715-545-020	1:50 1:200
2	CD163	GH1/61	AF532	Mouse IgG1, κ	BioLegend	Custom	1:200
	CD138	MI15	PE	Mouse IgG1, κ	BioLegend	356503	1:200
	HLA-DR	L243	iF594	Mouse IgG2a, κ	Caprico Biotechnologies	1032136	1:100
	CD49a	TS2/7	AF647	Mouse IgG1, κ	BioLegend	328310	1:200
	CD11c	B-Ly6	AF700	Mouse IgG1, κ	BD Biosciences	561352	1:25
	CD8	SK1	AF488	Mouse IgG1, κ	BioLegend	344716	1:25
	Vimentin	O91D3	AF532	Mouse IgG2a, κ	BioLegend	Custom	1:200

	CD31	WM59	PE	Mouse IgG1, κ	BioLegend	303106	1:100
	CD3	UCHT1	iF594	Mouse IgG1, κ	AAT Bioquest	103400C0	1:50
	CD4	RPA-T4	AF647	Mouse IgG1, κ	BioLegend	300520	1:100
	Ki-67	B56	AF700	Mouse IgG1, κ	BD Biosciences	561277	1:25
4	CD61	Y2/51	FITC	Mouse IgG1, κ	Miltenyi	130-098-682	1:25
	SPARC	Goat IgG	AF532	Goat IgG	R&D	AF941	1:50
	SMA-alpha	1A4	eF570	Mouse IgG2a, κ	Thermo	41-9760-82	1:300
	CD45	F10-89-4	iF594	Mouse IgG2a, κ	Caprico Biotechnologies	1016138	1:100
	CD44	IM7	AF647	Rat IgG2b, κ	BioLegend	103018	1:200
	Lumican	Polyclonal goat IgG	AF680	Goat IgG	R&D	AF2846	1:25
					Thermo	A-20188	

Human Liver (Manual IBEX): 22 parameters, 4 cycles

Cycle	Marker	Clone	Conjugate	Isotype	Vendor	Catalog Number	Dilution
1	Hoechst	-	-		Biotium	40046	1:5000
	Keratin 7	W16155A	AF488	Rat IgG2a, κ	BioLegend	601605	1:50
	ASS1	Polyclonal rabbit IgG Polyclonal donkey IgG	Donkey anti-rabbit IgG AF555	Rabbit IgG Donkey IgG	Abcam Thermo	Ab170952 A31572	1:100 1:300
	CD34	4H11	iF594	Mouse IgG1	AAT Bioquest	103400C0	1:50
	CD54	HA58	AF647	Mouse IgG1, κ	BioLegend	353114	1:50
	Lyve-1	Polyclonal goat IgG Polyclonal donkey IgG	Donkey anti-goat IgG AF555	Goat IgG Donkey IgG	R&D Thermo	AF2086-SP A21084	1:400 1:300
2	HLA-DR	L243	AF488	Mouse IgG2a, κ	BioLegend	307619	1:100
	CD163	GH1/61	AF532	Mouse IgG1, κ	BioLegend	99486	1:100
	CD138	MI15	PE	Mouse IgG1, κ	BioLegend	356503	1:200
	CD68	KP1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1064136	1:100
	Fibrinogen	Polyclonal sheep IgG Polyclonal donkey IgG	Donkey anti-sheep IgG AF680	Sheep IgG Donkey IgG	Abcam Thermo	Ab118533 A-21102	1:100 1:300
3	CD8	SK1	AF488	Mouse IgG1, κ	BioLegend	344716	1:25
	B-Tubulin 3	TUJ1	AF532	Mouse IgG2a, κ	BioLegend	Custom	1:100
	Glutamine Synthetase	Polyclonal rabbit IgG Anti-rabbit Fab	AF555	Rabbit IgG	Abcam Thermo	Ab49873 Z-25303	1:100
	CD3	UCHT1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1053134	1:50
	CD4	RPA-T4	AF647	Mouse IgG1, κ	BioLegend	300520	1:50
4	Ki-67	B56	AF700	Mouse IgG1, κ	BD Biosciences	561277	1:25
	αSMA	1A4	AF488	Mouse IgG2a, κ	Thermo	53-9760-80	1:600
	CD44	IM7	AF532	Rat IgG2b, κ	BioLegend	Custom	1:50
	CD49a	TS2/7	PE	Mouse IgG1, κ	BioLegend	328303	1:500
	CD45	HI30	AF594	Mouse IgG2a, κ	BioLegend	304060	1:100
	Vimentin	O91D3	AF647	Mouse IgG2a, κ	BioLegend	677807	1:600

Lymph node (Automated IBEX): 24 markers, 6 cycles

Cycle	Marker	Clone	Conjugate	Isotype	Vendor	Catalog Number	Dilution
1	Hoechst	-	-		Biotium	40046	1:5000
	CD20	L26	AF488	Mouse IgG2b, κ	eBioscience	53-0202-82	1:100
	CD34	4H11	iF594	Mouse IgG1	AAT Bioquest	103400C0	1:25
	CD54	HA58	AF647	Mouse IgG1, κ	BioLegend	353114	1:50
	Lyve-1	Polyclonal goat IgG		Goat IgG	R&D	AF2086-SP	1:400
	Collagen IV	Polyclonal rabbit IgG		Rabbit IgG	Abcam	Ab6586	1:50
2	Collagen IV	Donkey anti-rabbit IgG	AF488	Donkey IgG	Thermo	A-21206	1:200
	Lyve-1	Donkey anti-goat IgG	AF555	Donkey IgG	Thermo	A-21432	1:200
	CD68	KP1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1064135	1:50
	CD49a	TS2/7	AF647	Mouse IgG1, κ	BioLegend	328310	1:50
3	CD21	Bu32	AF488	Mouse IgG1, κ	BioLegend	98148	1:100
	CD35	E11	PE	Mouse IgG1, κ	BioLegend	333406	1:200
	HLA-DR	L243	iF594	Mouse IgG2a, κ	Caprico Biotechnologies	1032136	1:50
	CD163	GH11/61	AF647	Mouse IgG1, κ	BioLegend	333620	1:50
4	CD8	SK1	AF488	Mouse IgG1, κ	BioLegend	344716	1:25
	PD-1	EH12.2H7	PE	Mouse IgG1, κ	BioLegend	329906	1:50
	CD3	UCHT1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1053135	1:50
	CD4	RPA-T4	AF647	Mouse IgG1, κ	BioLegend	300520	1:25
5	Desmin	Y66	AF488	Rabbit IgG	Abcam	Ab185033	1:50
	CD31	WM59	PE	Mouse IgG1, κ	BioLegend	303106	1:100
	CD45	F10-89-4	iF594	Mouse IgG2a, κ	Caprico Biotechnologies	1016136	1:100
	CD138	MI15	AF647	Mouse IgG1, κ	BioLegend	356524	1:100
6	CD44	IM7	AF488	Rat IgG2b, κ	BioLegend	103016	1:50
	CD94	DX22	PE	Mouse IgG1, κ	BioLegend	305506	1:50
	Vimentin	O91D3	AF594	Mouse IgG2a, κ	BioLegend	677804	1:300
	αSMA	1A4	eF660	Mouse IgG2a, κ	Thermo	53-9760-82	1:200

Jejunum (Automated IBEX): 24 markers, 6 cycles

Cycle	Marker	Clone	Conjugate	Isotype	Vendor	Catalog Number	Dilution
1	Hoechst	-	-		Biotium	40046	1:5000
	EpCAM	9C4	AF488	Mouse IgG2b, κ	BioLegend	324210	1:300
	CD106	STA	PE	Mouse IgG1, κ	BioLegend	305806	1:50
	CD34	4H11	iF594	Mouse IgG1	AAT Bioquest	103400C0	1:25
	CD54	HA58	AF647	Mouse IgG1, κ	BioLegend	353114	1:50
	Lysozyme	Polyclonal rabbit IgG		Rabbit IgG	Abcam	Ab2408	1:50
	Lyve-1	Polyclonal goat IgG		Goat IgG	R&D	AF2089	1:100
2	Lysozyme	Donkey anti-rabbit IgG	AF488	Donkey IgG	Thermo	A-21206	1:200
	Lyve-1	Donkey anti-goat IgG	AF555	Donkey IgG	Thermo	A-21432	1:200
	CD68	KP1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1064135	1:50
	CD49a	TS2/7	AF647	Mouse IgG1, κ	BioLegend	328310	1:50
3	Tubulin3	TUJ1	AF488	Mouse IgG2a, κ	BioLegend	801203	1:50
	CD31	WM59	PE	Mouse IgG1, κ	BioLegend	303106	1:100
	HLA-DR	L243	iF594	Mouse IgG2a, κ	Caprico Biotechnologies	1032136	1:50
	Cytokeratin	AE1/AE3	eF660	Mouse IgG1	Thermo	50-9003-82	1:50
4	CD8	SK1	AF488	Mouse IgG1, κ	BioLegend	344716	1:25
	PD-1	EH12.2H7	PE	Mouse IgG1, κ	BioLegend	329906	1:50
	CD3	UCHT1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1053135	1:50
	CD4	RPA-T4	AF647	Mouse IgG1, κ	BioLegend	300520	1:25
5	IgA2	A9604D2	AF488	Mouse IgG1, κ	SouthernBiotech	9140-30	1:400
	CD138	MI15	PE	Mouse IgG1, κ	BioLegend	356503	1:100
	CD45	F10-89-4	iF594	Mouse IgG2a, κ	Caprico Biotechnologies	1016136	1:100
	IgA1	B3506B4	AF647	Mouse IgG1, κ	SouthernBiotech	9130-31	1:400
6	CD44	IM7	AF488	Rat IgG2b, κ	BioLegend	103016	1:100
	αSMA	1A4	eF570	Mouse IgG2a, κ	Thermo	41-9760-82	1:200
	Vimentin	O91D3	AF647	Mouse IgG2a, κ	BioLegend	677807	1:300

Skin (Automated IBEX): 19 markers, 5 cycles

Cycle	Marker	Clone	Conjugate	Isotype	Vendor	Catalog Number	Dilution
1	Hoechst	-	-		Biotium	40046	1:5000
	CD34	4H11	iF594	Mouse IgG1	AAT Bioquest	103400C0	1:25
	CD54	HA58	AF647	Mouse IgG1, κ	BioLegend	353114	1:50
	Lumican	Polyclonal goat IgG		Goat IgG	R&D	AF2846	1:200
	EpCAM	Polyclonal rabbit IgG		Rabbit IgG	Abcam	Ab71916	1:50
2	Lumican	Donkey anti-goat IgG	AF488	Donkey IgG	Thermo	A-11055	1:400
	EpCAM	Donkey anti-rabbit IgG	AF555	Donkey IgG	Thermo	A-31572	1:200
	CD68	KP1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1064135	1:50
	CD49a	TS2/7	AF647	Mouse IgG1, κ	BioLegend	328310	1:50
	Langerin	929F3.01		Rat IgG2a	Novus	DDX0362P-100	1:50
3	HLA-DR	L243	AF488	Mouse IgG2a, κ	BioLegend	307620	1:50
	CD117	104D2	PE	Mouse IgG1, κ	BioLegend	313204	1:50
	CD3	UCHT1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1053135	1:25
	Langerin	Donkey anti-Rat IgG	AF647	Donkey IgG	Jackson Immuno Research	712-605-153	1:200
4	CD44	IM7	AF488	Rat IgG2b, κ	BioLegend	103054	1:50
	CD31	WM59	PE	Mouse IgG1, κ	BioLegend	303106	1:100
	CD45	F10-89-4	iF594	Mouse IgG2a, κ	Caprico Biotechnologies	1016136	1:100
	CD138	MI15	AF647	Mouse IgG1, κ	BioLegend	356524	1:100
	Keratin 14	Poly9060		Chicken IgY	BioLegend	906004	1:50
5	Keratin 10	DE-K10	AF488	Mouse IgG1, κ	Novus	NBP2-54402AF488	1:200
	Keratin 14	Goat anti-Chicken IgY	AF555	Goat IgG	Invitrogen	A32932	1:400
	Vimentin	O91D3	AF594	Mouse IgG2a, κ	BioLegend	677807	1:300
	αSMA	1A4	eF660	Mouse IgG2a, κ	Thermo	41-9760-82	1:50

Kidney (Automated IBEX, FFPE): 16 markers, 5 cycles

Cycle	Marker	Clone	Conjugate	Isotype	Vendor	Catalog Number	Dilution
1	Hoechst	-	-		Biotium	40046	1:5000
	Cathepsin L	204101		Rat IgG2a	R&D	MAB-9521	1:50
	CD10	Polyclonal goat IgG		Goat IgG	R&D	AF1182	1:200
	Podocin	JB51-33		Rabbit IgG	Novus	NBP2-75624	1:50
	Cytokeratin	AE1/AE3	AF488	Mouse IgG1	Thermo	53-9003-82	1:50
	CD34	QBEND-10	PE	Mouse IgG1, κ	Thermo	MAI-10205	1:50
	CD68	KP1	iF594	Mouse IgG1, κ	Caprico Biotechnologies	1064135	1:25
	αSMA	1A4	eF660	Mouse IgG2a, κ	Thermo	50-9760-82	1:50
	CD10	Donkey anti-goat IgG	AF488	Donkey IgG	Thermo	A-11055	1:400

	Podocin	Donkey anti-rabbit IgG	AF555	Donkey IgG	Thermo	A-21432	1:400
	CD66b	G10F5	iF594	Mouse IgM, κ	Caprico Biotechnologies	1075139	1:25
	Cathepsin L	Donkey anti-rat IgG	AF647	Rat IgG2a	Jackson ImmunoResearch	712-605-153	1:100
	CD15	MMA		Mouse IgM, κ	BD	347420	1:25
3	CD15 Donkey anti-mouse IgM AF488	Polyclonal	AF488	Donkey IgG	Jackson ImmunoResearch	715-545-020	1:200
	ACE2	Polyclonal, Custom conjugate	AF555	Goat IgG	R&D Abcam	AF933 Ab269820	1:50
	SPARC	Polyclonal, Custom conjugate	iF594	Goat IgG	R&D AAT Bioquest	AF941 1230	1:50
	TMPRSS2	EPR3861, Custom conjugate	AF647	Rabbit IgG	Abcam Abcam	Ab92323 Ab269823	1:50
4	Lumican iF594	Polyclonal goat IgG Custom conjugate	iF594	Goat IgG	R&D AAT Bioquest	AF2846 1230	1:200
5	Uromodulin	10.32	FITC	Mouse IgG2b	Novus	NBP1-50431	1:25
	Glycophorin	EPR8200, Custom conjugate	AF555	Rabbit IgG	Abcam Abcam	Ab218372 Ab269820	1:300
	Syndecan-1	Polyclonal, Custom conjugate	iF594	Goat IgG	R&D AAT Bioquest	AF2780 1230	1:50
	Vimentin	O91D3	AF647	Mouse IgG2a, κ	BioLegend	677807	1:200

Complete antibody metadata for tissue-specific panels and accompanying imaging data can be found here: <https://zenodo.org/record/5244551#.YSXTRIhKguV>

Please see original IBEX publication for additional panels including the following mouse tissues: lymph node, spleen, thymus, lung, small intestine, and liver: <https://doi.org/10.1073/pnas.2018488117>.

Table S3. Recommendations for optimal antibody and fluorophore pairing for IBEX panel design.

Ex/Em Max (nm)	Fluorophore	Inactivation Method	Dim (1)-Brightest (5)	IBEX Method	Special Considerations
350/461	Hoechst 33342	-	5	Both	Preferred fiducial for registration and nuclear segmentation Overcomes high autofluorescence in this channel, especially in human tissues
410/455	Pacific Blue	<15 minutes	1	Manual (tested)	Dim, limited availability of direct conjugates Use Hoechst 33342 in this channel
405/421	Brilliant Violet 421	<15 minutes + Light	4	Manual (tested)	Use Hoechst 33342 in this channel High autofluorescence in human tissues Light inactivation is tedious for large areas
405/510	Brilliant Violet 510	<15 minutes + Light	1	Manual (tested)	Use Hoechst 33342 in this channel High autofluorescence in human tissues Light inactivation is tedious for large areas
490/525	AF488	<15 minutes	4	Both	Excellent fluorophore for IBEX Wide availability of direct conjugates, labeling kits, and secondary antibodies Pair with moderate to highly expressed markers
490/525	FITC	<30 minutes	3	Both	Prioritize AF488 over FITC conjugated antibodies
532/554	AF532	<15 minutes	3	Manual	Suitable fluorophore for manual IBEX Limited availability of direct conjugates and secondary antibodies Pair with highly expressed markers and be mindful of spectral overlap between AF555/PE/eF570 channel
533/544	JOJO-1	-	4	Manual	Avoid and use Hoechst 33342 as nuclear marker and fiducial instead
555/580	AF555	<15 minutes	3	Both	Excellent fluorophore for IBEX Wide availability of direct conjugates, labeling kits, and secondary antibodies Pair with moderate to highly expressed markers and be mindful of spectral overlap with AF532 channel
546/585	eF570	<15 minutes	3	Both	Good fluorophore for IBEX Limited availability of direct conjugates, labeling kits, and secondary antibodies Pair with moderate to highly expressed markers and be mindful of spectral overlap with AF532 channel
488-561/565	PE	<15 minutes	5	Both	Prioritize AF555 and eF570 conjugates Prone to photobleaching but can be paired with dim markers Be mindful of spectral overlap with AF532 channel and non-specific binding to tissues
587/603	iF594	<15 minutes	4	Both	Excellent fluorophore for IBEX Limited availability of direct conjugates, labeling kits, and secondary antibodies
590/617	AF594	-	5	Both	Can be paired with a structural marker and used as fiducial or placed in the final IBEX cycle Avoid and use Hoechst 33342 as fiducial and iF594 instead
560/645	eF615	-	4	Both	Can be paired with a structural marker and used as fiducial or placed in the final IBEX cycle Avoid and use Hoechst 33342 as fiducial and iF594 instead Limited availability of direct conjugates, labeling kits, and secondary antibodies
650/665	AF647	<15 minutes	5	Both	Excellent fluorophore for IBEX Wide availability of direct conjugates, labeling kits, and secondary antibodies Pair with dim to moderately expressed markers
633/669	eF660	<15 minutes	4	Both	Excellent fluorophore for IBEX Limited availability of direct conjugates, labeling kits, and secondary antibodies Pair with dim to moderately expressed markers
679/702	AF680	<15 minutes	3	Manual	Suitable fluorophore for manual IBEX, cannot be separated from adjacent channels on THUNDER Limited availability of direct conjugates and secondary antibodies Pair with highly expressed markers
702/723	AF700	<15 minutes	1	Manual	Suitable fluorophore for manual IBEX Limited availability of direct conjugates and secondary antibodies Pair with highly expressed markers and place in Cycles 1-4
749/775	AF750	<15 minutes	3	Automated	Suitable fluorophore for automated IBEX on THUNDER but signal greatly diminished with AFC, in general avoid Limited availability of direct conjugates and secondary antibodies

Table S4. Estimated costs for IBEX implementation.

Tissue Grossing and Processing • Reagents and consumables for 100 samples <\$5,000 • Tools for embedding/processing for 100 samples <\$1,000 • Cryostat (Leica CM1950) or equivalent, available in most core facilities ~\$45,000 • Rotary microtome (Leica, RM2255) or equivalent, available in most core facilities <\$20,000 • Stereomicroscope and illuminator <\$5,000	Manual and Automated IBEX
Immunolabeling and microscopy • Leica TCS SP8 X confocal microscope or equivalent, highly variable \$500,000-700,000 • PELCO BioWave Pro microwave and SteadyTemp Pro, optional (incubate at 37°C) ~\$31,000 • ~\$4 per antibody per run (2 µg in 400 µl), scales with complexity of panels and number of samples	Manual IBEX
Immunolabeling and microscopy • Leica THUNDER microscope or equivalent, dependent on configuration \$175,000-200,000 • ARIA Fluidics Device <\$15,000 • Imaging chamber, vacuum, and heater from Warner instruments <\$5,000 • Additional accessories (triggering cables) <\$100 • ~\$4 per antibody per run (2 µg in 400 µl), scales with complexity of panels and number of samples	Automated IBEX
Software and system requirements for image processing • Imaris and Imaris File Converter (x64, version 9.5.0), variable depending on bulk pricing and bundling with other licenses, \$5,000-10,000 per license • Imaris Viewer \$0 • Custom Imaris Extension \$0 • System requirements for image processing, highly variable \$4,000-30,000 ◦ MacBook Pro 2.3 GHz 8-Core i9 with 32 GB memory \$4,000, scaled to multiple nodes on a high-performance cluster (>\$5,000 per node) for batch processing ◦ HP Z8 G4 Workstation or equivalent \$16,000-21,000	Manual and Automated IBEX

Videos S1-S7 Legends

Video S1. High dimensional imaging of human lymph node using manual IBEX method. Confocal images of human mesenteric lymph node from a 9 cycle 38 parameter IBEX experiment with Hoechst serving as a fiducial.

Video S2. High dimensional imaging of human spleen using manual IBEX method. Confocal images of human spleen from a 4 cycle 25 parameter IBEX experiment with Hoechst serving as a fiducial.

Video S3. High dimensional imaging of human liver using manual IBEX method. Confocal images of human liver from a 4 cycle 22 parameter IBEX experiment with Hoechst serving as a fiducial.

Video S4. High dimensional imaging of human lymph node using automated IBEX method. THUNDER widefield images of human mesenteric lymph node from a 6 cycle 24 parameter IBEX experiment with Hoechst serving as a fiducial.

Video S5. High dimensional imaging of human jejunum using automated IBEX method. THUNDER widefield images of human jejunum from a 6 cycle 24 parameter IBEX experiment with Hoechst serving as a fiducial.

Video S6. High dimensional imaging of human skin using automated IBEX method. THUNDER widefield images of human skin from a 5 cycle 19 parameter IBEX experiment with Hoechst serving as a fiducial.

Video S7. High dimensional imaging of human kidney (FFPE) using automated IBEX method. THUNDER widefield images of human FFPE kidney sections from a 5 cycle 16 parameter IBEX experiment with Hoechst serving as a fiducial.