BioLegend®

The path to legendary discovery™

LEGENDScreen™

Lyophilized Antibody Panel

Human Cell Screening (PE) Kit

Catalog Number:	700001
Size:	1 kit, ready to use. 1 test per antibody/well
Reactivity:	Human
Antibody Format:	PE-conjugated
Configuration:	342 pre-titrated antibodies, including 10 isotype controls, arrayed on four 96-well plates, with one specificity per well.

It is highly recommended that this manual be read in its entirety before using this product.
Do not use this kit beyond the expiration date.

For research use only

BioLegend, Inc.
biolegend.com

Rev. 121212

Table of Contents Page

Description

The recent advances in stem cell research, such as hematopoietic and cancer stem cell research, have opened the doors for treating diseases that were difficult to treat before, including cancer and autoimmune diseases. Individual fluorochrome-conjugated antibodies or antibody cocktails have been used as powerful tools for immunology research. However, there is an increasing need for tools that can quickly screen cells for a large number of cell surface markers. The BioLegend LEGENDScreen™ Human Cell Screening (PE) Kit is made to meet this need.

This kit contains 332 PE-conjugated monoclonal antibodies to cell surface markers as well as 10 mouse, rat, or Armenian hamster Ig isotype controls. All of the antibodies have been pre-titrated to optimal concentrations, arrayed on four 96-well plates, and lyophilized in a ready-to-use format. This kit can be used to screen cell lines and primary cells (PBMCs, bone marrow derived cells, and cells isolated from tissues). There are blank wells on the plates for users to add their choice of markers. The end user can also choose to screen cells of interest directly or co-stain with other markers labeled with different fluorochromes. Positive "hits" from the screening can be quickly identified based on the plate map, and the catalog number and clone information can be obtained using the tables attached. Analysis with individual fluorochrome-conjugated antibodies should be performed to confirm the screening results.

The LEGENDScreen™ Human Cell Screening (PE) Kit provides a convenient, easy to use, and powerful tool for immunology, stem cell, and cancer research.

Materials Provided

Description	Quantity	Volume	Part #
Human Cell Screening (PE) Kit Plate #1	**1 plate**		**77046**
Human Cell Screening (PE) Kit Plate #2	**1 plate**		**77047**
Human Cell Screening (PE) Kit Plate #3	**1 plate**		**77048**
Human Cell Screening (PE) Kit Plate #4	**1 plate**		**77049**
Cell Staining Buffer	**1 bottle**	**500 mL**	**420201**
Fixation Buffer	**1 bottle**	**100 mL**	**420801**
Plate Sealers	**12 sheets**		**78101**

Tel: 858-768-5800

Materials to be Provided by the End User

1. Adjustable multichannel pipettes for measuring volumes ranging from 25 µL to 1,000 µL
2. Centrifuge with a rotor and adaptors for 96-well plates
3. Deionized water
4. Cell culture medium
5. Cell dissociation buffer (for adherent cells)
6. 1X PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered
7. Plastic reservoirs for pipetting deionized water or wash buffer with a multichannel pipette
8. A flow cytometer, preferably compatible with reading 96-well plates

Storage and Handling

1. Store unopened kit components at 4°C. Do not use this kit beyond its expiration date.
2. Once opened, plates should be reconstituted in deionized water immediately. The reconstituted plates can be used or sealed for stroage at 4°C in the dark for up to one month.
3. The buffers should be kept at 4°C after opening and used within one month.

Preparation of Cells for Staining

1. Obtain blood or desired tissue (*e.g. spleen, lymph node, thymus, bone marrow*) and prepare a single cell suspension. Wash cells in 1X PBS or cell culture medium of choice, and resuspend in Cell Staining Buffer at a density between 4×10^6 and 1×10^7 cells/mL.
2. For cultured cells in suspension, spin and resuspend cells in Cell Staining Buffer at a density between 4×10^6 and 1×10^7 cells/mL.
3. For cultured adherent cells, dissociate cells using a mild enzyme (*e.g. StemPro® Accutase® Cell Dissociation Reagent, Cat. No. A11105-01, Invitrogen*) or non-enzymatic dissociation buffer (*Cell Dissociation Buffer, enzyme-free, PBS, Cat. No. 13151-014, Invitrogen*). Wash cells in 1X PBS or cell culture medium of choice, and resuspend in Cell Staining Buffer at a density between 4×10^6 and 1×10^7 cells/mL.
4. Filter the cells through a 40 µm cell strainer to remove any clumps. Keep the cells on ice before use. Approximately 30 mL of cells at a density between 4×10^6 to 1×10^7 cells/mL is needed for all four plates.
5. Optional: Reagents that block Fc receptors may be useful for reducing nonspecific immunofluorescent staining. In the absence of an effective/

available blocking antibody for human Fc receptors, an alternative approach is to pre-block cells with excessive irrelevant purified Ig from the same species and isotype as the antibodies used for immunofluorescent staining.

Plate Preparation

One hour before the staining, perform the following steps to prepare the plates.

1. Remove the lyophilized plates from the aluminum pouches.
2. Centrifuge the plates at 600 × *g* for 5 minutes.
3. Check and make sure the lyophilized cakes settled to the bottom of the plates. Keep the plates upright at all times from this point forward. Handle plates with care so that the cakes are not agitated at any time.
4. Gently settle the plates on the bench. **Hold the plate firmly and carefully remove the plate cover from one corner, one well at a time, for easy opening and preventing cross-well contamination.** You may need to wiggle the cover a little while pulling back slowly.
5. **Discard the cover. Do not reseal with the original cover or apply any sealer until the lyophilized antibodies have been reconstituted.**
6. Reconstitute the lyophilized antibodies **immediately**, with 25 µL/well of deionized water using a multichannel pipette. To avoid cross-contamination, do not let the pipette tips touch the wells. Make sure that all the cakes are dissolved in water. If a cake is stuck on the side of the well, pipette the 25 µL water added to the well and rinse the cake down to the bottom.
7. Seal the plates with the plate sealers provided.
8. Wait at least 15 minutes before the staining procedure. Keep the plates in the dark.

Note:

- PE-conjugated antibodies are light sensitive. Try to minimize the exposure of the plates to light as much as practically possible.
- Do not open the pouches until the day you are ready to run the experiment. Once the plates are removed from the pouches, the antibodies must be reconstituted immediately.
- If an experiment is not performed after reconstitution, plates can be sealed and stored in the dark at 4°C for up to one month.

Cell Staining Procedure

1. Using a multichannel pipette, add 75 µL/well of cells to the plates as prepared above.
2. Set up extra tubes to stain cells for flow cytometer setup and compensation, if needed.

3. Using the multichannel pipette, gently mix the cell suspensions by pipetting up and down 2 - 3 times. **Be sure to change tips between each row**. Avoid creating bubbles while pipetting.
4. Incubate for 20 - 30 minutes at 4°C in the dark.
5. Spin the plates at 500 × *g* for 6 minutes to pellet cells in each well. Immediately after centrifugation, dump the supernatant into the sink by quickly inverting and flicking the plate. Gently blot the plate on a paper towel, being careful not to disturb the cell pellet.
6. Use a multi-channel pipette to deliver 200 µL of Cell Staining Buffer to each well. Gently mix up and down to resuspend cells. **Be sure to change tips between each row.**
7. Repeat step 5.
8. To fix the cells, using a multichannel pipette, aliquot 100 µL of Fixation Buffer into each well. Gently mix up and down to resuspend cells. **Be sure to change tips between each row**.
9. Incubate for 10 minutes at room temperature in the dark.
10. Repeat steps 5 - 6.
11. Repeat step 5 one more time.
12. Resuspend cells completely in 160 µL of Cell Staining Buffer per well and analyze using a flow cytometer. We recommend analyzing 70 µL of samples and collecting at least 5,000 events, but the end user may need to determine the optimal number of events to be collected. While the first plate is being analyzed, store the other plates at 4°C in the dark.

Tips for a Successful Staining

1. Read the entire manual carefully before the experiment.
2. Plan the experiement in advance. Designate a full day for this experiment. Do not rush any step.
3. Make sure that the flow cytometer's autosampler is well maintained and working well before the experiment. If the flow cytometer does not have an autosampler, the samples from each well of the plates should be transferred to individual FACS tubes and read manually. In this case, the volume of the samples may need to be increased to avoid running dry.
4. Make sure that enough cells have been prepared for the staining. If there are not enough cells, you may choose to separate the staining into two separate experiments (*e.g. stain two plates each time*).
5. Make sure to prepare cells for machine set up and compensation. These cells should be treated the same way as the cells for staining in the plates.
6. Handle the plates with care. Keep the plates upright at all times and be careful not to knock the plates over.

7. Protect the plates from exposure to light as much as possible.
8. Use extra caution when opening the plate cover (**see Plate Preparation for instructions**). After the cover is removed, do not apply any sealers on the plates until the cakes are reconstituted. Applying plate sealers or any cover onto the plate before the cakes are reconstituted can result in cakes being dislodged and stuck to the cover or escaping from the wells.
9. Some cell surface markers are sensitive to enzymatic digestion. If adherent cells are being used for staining, a mild enzyme or non-enzymatic dissociation buffer should be used when possible.
10. Make sure cells are in a single cell suspension. DNase treatment is recommended to avoid clumps caused by dead cells.
11. Analyze only 70 µL of the 160 µL samples so that a second run can be performed if necessary.

Frequently Asked Questions

Q: What is the level of variability from one experiment to the other?

A: If the protocol is followed closely, the variability should be minimal. The variability should be similar to single vial antibody staining.

Q: How should the kit be stored?

A: The kit should be stored at 2 - 8°C upon receipt. Once opened, the plates must be reconstituted immediately. Reconstituted plates can be used or stored at 2 - 8°C sealed in the dark and used within a month.

Q: How do I request a custom LEGENDScreen™ product with only my specificities of interest?

A: For more info, visit: **biolegend.com/custom_solutions**

Q: What are the guarantees regarding the lyophilized plate compared to the reconstituted plate?

A: Lyophilized product has a guaranteed shelf life of 6 months unopened. Reconstituted plates can be used or stored at 2 - 8°C sealed in the dark and used within a month. Be sure to properly seal the plates to prevent evaporation and shield the antibodies from light.

Q: I have added my own antibody solution to the lyophilized product, will the lyophilized antibody work?

A: Yes, as long as the fluorophores on these antibodies are compatible and proper compensation has been applied during acquisition and analysis.

Q: I am not going to use all the reconstituted antibody solution. Can I keep the left over for later or re-dry the solution in dark?

A: The antibody is in a one test per well format. There will not be any antibody left if the full test is used. Customers may decide to use less than the recommended volume per test, but this is not recommended and the performance is not guaranteed. Customers may also selectively transfer certain antibodies from the original plate to a new plate and use after reconstitution. If any antibody is not used after reconstitution, the plate can be sealed and store at 2 - 8°C for a month in the dark. Once reconstituted, re-drying is not recommended as this may result in a loss of signal.

Q: If I don't have enough cells and use much less than 4 million/ml, will it still work?

A: This may work with lower numbers of total cells, but we recommend trying to keep higher concentrations of cells for faster analysis. Of course, how many cells are needed depends on the specific application.

Q: Are these plates made under sterile conditions?

A: The plates are not sterile. You would handle them as you would handle most typical flow cytometry staining protocols.

Q: Can I use half or less of the plate and keep the rest for later?

A: Yes. Customers can use half of the plate or whatever specificities they are interested in. However, the whole plate should be reconstituted. The half plate of antibodies must be transferred to another empty plate for the staining. The remaining half must be sealed and stored at 2 - 8°C in the dark and used within a month.

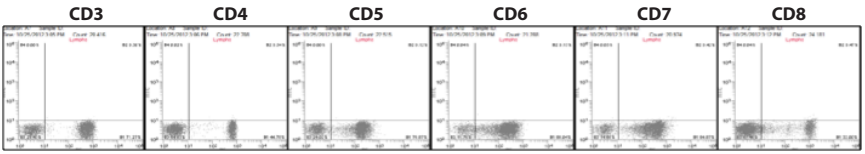
Product Performance

The LEGENDScreen™ Human Cell Screening (PE) Kit was tested and compared with BioLegend's cataloged single vial liquid antibody reagents. For cell staining, PBMCs were isolated using Ficoll-Paque™ Plus (GE Healthcare) and 0.25×10^6 cells were added to each well after the lyophilized antibodies were reconstituted. The cells were then stained for 20 minutes at 4 °C, washed, and fixed with Fixation Buffer. The cells were then washed, resuspended in 160 µL of Cell Staining Buffer, and analyzed using an iCyt Eclipse Flow Cytometry Analyzer.

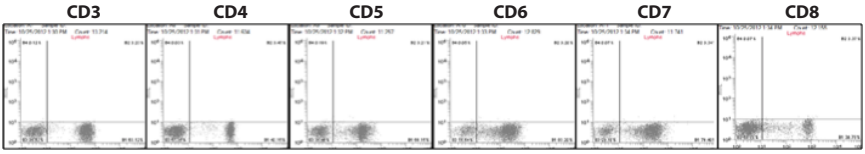
There were no significant differences in the staining patterns or median fluorescence intensity between the lyophilized product and liquid antibodies. Below are the representative typical data obtained when comparing the LEGENDScreen™ Human Cell Screening (PE) Kit vs. Cataloged liquid antibodies.

Representative Data:

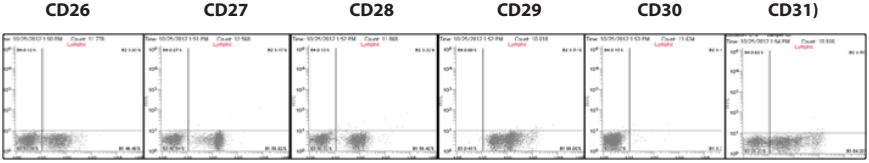
Catalog Single Vial



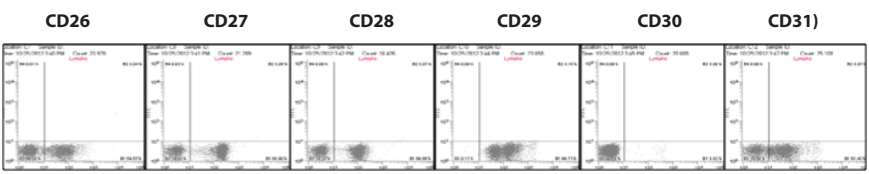
LEGENDScreen™



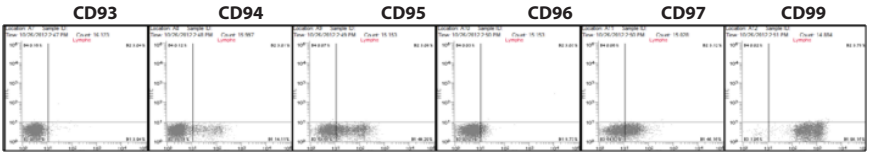
Catalog Single Vial



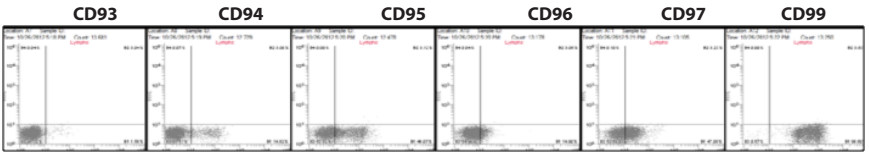
LEGENDScreen™



Catalog Single Vial

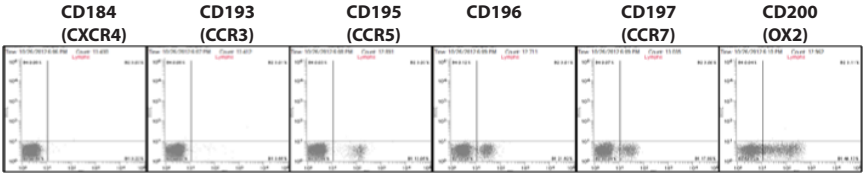


LEGENDScreen™

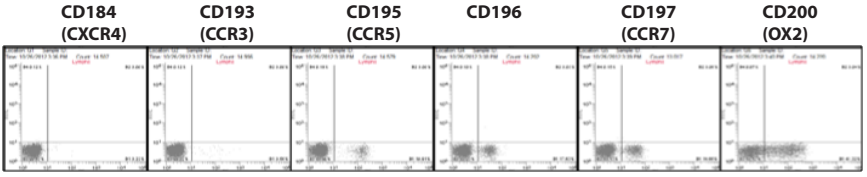


Tel: 858-768-5800

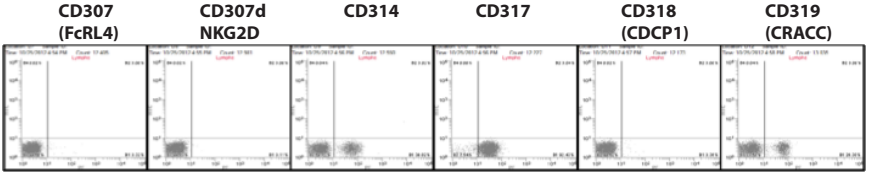
Catalog Single Vial



LEGENDScreen™



Catalog Single Vial



LEGENDScreen™

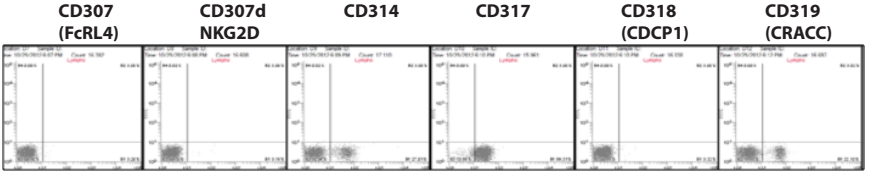


Plate Maps:

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	CD1a	CD1b	CD1c	CD1d	CD2	CD3	CD4	CD5	CD6	CD7	CD8a
B	CD9	CD10	CD11a	CD11b	CD11b (activated)	CD11c	CD13	CD14	CD15	CD16	CD18	CD19
C	CD20	CD21	CD22	CD23	CD24	CD25	CD26	CD27	CD28	CD29	CD30	CD31
D	CD32	CD33	CD34	CD35	CD36	CD38	CD39	CD40	CD41	CD42b	CD43	CD44
E	CD45	CD45RA	CD45RB	CD45RO	CD46	CD47	CD48	CD49a	CD49c	CD49d	CD49e	CD49f
F	CD50 (ICAM-3)	CD51	CD51/61	CD52	CD53	CD54	CD55	CD56 (NCAM)	CD57	CD58	CD59	CD61
G	CD62E	CD62L	CD62P (P-Selectin)	CD63	CD64	CD66a/c/e	CD66b	CD69	CD70	CD71	CD73	CD74
H	CD79b	CD80	CD81	CD82	CD83	CD84	CD85a (ILT5)	CD85d (ILT4)	CD85g (ILT7)	CD85h (ILT1)	CD85j (ILT2)	CD85k (ILT3)

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	CD86	CD87	CD88	CD89	CD90 (Thy1)	CD93	CD94	CD95	CD96	CD97	CD99
B	CD100	CD101 (BB27)	CD102	CD103	CD104	CD105	CD106	CD107a (LAMP-1)	CD108	CD109	CD111	CD112 (Nectin-2)
C	CD114	CD115	CD116	CD117 (c-kit)	CD119 (IFN-γ R α chain)	CD122	CD123	CD124	CD126 (IL-6Rα)	CD127 (IL-7Rα)	CD129 (IL-9 R)	CD131
D	CD132	CD134	CD135	CD137 (4-1BB)	CD137L (4-1BB L)	CD138	CD140a	CD140b	CD141	CD143	CD144	CD146
E	CD148	CD150 (SLAM)	CD152	CD154	CD155 (PVR)	CD156c (ADAM-10)	CD158a/h	CD158b (NKAT2)	CD158d	CD158e1 (NKB1)	CD158f	CD161
F	CD162	CD163	CD164	CD165	CD166	CD167a (DDR1)	CD169	CD170 (Siglec-5)	CD172a (SIRPa)	CD172b (SIRPb)	CD172g (SIRPg)	CD178 (Fas-L)
G	CD179a	CD179b	CD180 (RP105)	CD181 (CXCR1)	CD182 (CXCR2)	CD183	CD184 (CXCR4)	CD193 (CCR3)	CD195 (CCR5)	CD196	CD197 (CCR7)	CD200 (OX2)
H	CD200 R	CD201 (EPCR)	CD202b (Tie2/Tek)	CD203c (E-NPP3)	CD205 (DEC-205)	CD206 (MMR)	CD207 (Langerin)	CD209 (DC-SIGN)	CD210 (IL-10 R)	CD213a2	CD215 (IL-15Rα)	CD218a (IL-18Rα)

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	CD220	CD221 (IGF-1R)	CD226 (DNAM-1)	CD229 (Ly-9)	CD231 (TALLA)	CD235ab	CD243	CD244 (2B4)	CD245 (p220/240)	CD252 (OX40L)	CD253 (Trail)
B	CD254	CD255 (TWEAK)	CD257 (BAFF)	CD258 (LIGHT)	CD261 (DR4)	CD262 (DR5,)	CD263 (DcR1)	CD266 (Fn14)	CD267 (TACI)	CD268 (BAFF-R)	CD270 (HVEM)	CD271
C	CD273 (B7-DC)	CD274 (B7-H1)	CD275 (B7-H2)	CD276	CD277	CD278 (ICOS)	CD279 (PD-1)	CD282 (TLR2)	CD284 (TLR4)	CD286 (TLR6)	CD290	CD294
D	CD298	CD300e (IREM-2)	CD300F	CD301	CD303	CD304	CD307	CD307d (FcRL4)	CD314 (NKG2D)	CD317	CD318 (CDCP1)	CD319 (CRACC)
E	CD324 (E-Cadherin)	CD325	CD326 (Ep-CAM)	CD328 (Siglec-7)	CD334 (FGFR4)	CD335 (NKp46)	CD336 (NKp44)	CD337 (NKp30)	CD338 (ABCG2)	CD340 (erbB2)	CD344 (Frizzled-4)	CD351
F	CD352 (NTB-A)	CD354 (TREM-1)	CD355 (CRTAM)	CD357 (GITR)	CD360 (IL-21R)	β2-micro-globulin	BTLA	C3AR	C5L2	CCR10	CLEC12A	CLEC9A
G	CX3CR1	CXCR7	δ-Opioid Receptor	DLL1	DLL4	DR3 (TRAMP)	EGFR	erbB3/ HER-3	FceRIa	FcRL6	Galectin-9	GARP (LRRC32)
H	HLA-A,B,C	HLA-A2	HLA-DQ	HLA-DR	HLA-E	HLA-G	IFN-γ R b chain	Ig light chain k	Ig light chain λ	IgD	IgM	IL-28RA

Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Integrin α9β1	integrin β5	integrin β7	Jagged 2	LAP	LT-β R	Mac-2 (Galec-tin-3)	MAIR-II	MICA/ MICB	MSC (W3D5)	MSC (W5C5)
B	MSC (W7C6)	MSC and NPC (W4A5)	MSCA-1 (MSC)	NKp80	Notch 1	Notch 2	Notch 3	Notch 4	NPC (57D2)	Podopla-nin	Pre-BCR	PSMA
C	Siglec-10	Siglec-8	Siglec-9	SSEA-1	SSEA-3	SSEA-4	SSEA-5	TCR g/d	TCR Vβ13.2	TCR Vβ23	TCR Vβ8	TCR Vβ9
D	TCR Vδ2	TCR Vg9	TCR Vα24-Jα18	TCR Vα7.2	TCR α/β	Tim-1	Tim-3	Tim-4	TLT-2	TRA-1-60-R	TRA-1-81	TSLPR (TSLP-R)
E	Ms IgG1, κ ITCL	Ms IgG2a, κ ITCL	Ms IgG2b, κ ITCL	Ms IgG3, κ ITCL	Ms IgM, κ ITCL	Rat IgG1, κ ITCL	Rat IgG2a, κ ITCL	Rat IgG2b, κ ITCL	Rat IgM, κ ITCL	AH IgG, ITCL	Blank	Blank
F	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
G	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

Antibody Information Tables

Plate 1

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
A1	Blank			
A2	CD1a	HI149	Mouse IgG1, κ	300106
A3	CD1b	SN13 (K5-1B8)	Mouse IgG1, κ	329108
A4	CD1c	L161	Mouse IgG1, κ	331506
A5	CD1d	51.1	Mouse IgG2b, κ	350306
A6	CD2	RPA-2.10	Mouse IgG1, κ	300208
A7	CD3	HIT3a	Mouse IgG2a, κ	300308
A8	CD4	RPA-T4	Mouse IgG1, κ	300508
A9	CD5	UCHT2	Mouse IgG1, κ	300608
A10	CD6	BL-CD6	Mouse IgG1, κ	313906
A11	CD7	CD7-6B7	Mouse IgG2a, κ	343106
A12	CD8a	HIT8a	Mouse IgG1, κ	300908
B1	CD9	HI9a	Mouse IgG1, κ	312106
B2	CD10	HI10a	Mouse IgG1, κ	312204
B3	CD11a	HI111	Mouse IgG1, κ	301208
B4	CD11b	ICRF44	Mouse IgG1, κ	301306
B5	CD11b (activated)	CBRM1/5	Mouse IgG1, κ	301406
B6	CD11c	3.9	Mouse IgG1, κ	301606
B7	CD13	WM15	Mouse IgG1, κ	301704
B8	CD14	M5E2	Mouse IgG2a, κ	301806
B9	CD15 (SSEA-1)	W6D3	Mouse IgG1, κ	323006
B10	CD16	3G8	Mouse IgG1, κ	302008
B11	CD18	TS1/18	Mouse IgG1, κ	302108
B12	CD19	HIB19	Mouse IgG1, κ	302208
C1	CD20	2H7	Mouse IgG2b, κ	302306
C2	CD21	Bu32	Mouse IgG1, κ	354904
C3	CD22	HIB22	Mouse IgG1, κ	302506

Plate 1

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
C4	CD23	EBVCS-5	Mouse IgG1, κ	338508
C5	CD24	ML5	Mouse IgG2a, κ	311106
C6	CD25	BC96	Mouse IgG1, κ	302606
C7	CD26	BA5b	Mouse IgG2a, κ	302706
C8	CD27	O323	Mouse IgG1, κ	302808
C9	CD28	CD28.2	Mouse IgG1, κ	302908
C10	CD29	TS2/16	Mouse IgG1, κ	303004
C11	CD30	BY88	Mouse IgG1, κ	333906
C12	CD31	WM59	Mouse IgG1, κ	303106
D1	CD32	FUN-2	Mouse IgG2b, κ	303206
D2	CD33	WM53	Mouse IgG1, κ	303404
D3	CD34	581	Mouse IgG1, κ	343506
D4	CD35	E11	Mouse IgG1, κ	333406
D5	CD36	5-271	Mouse IgG2a, κ	336206
D6	CD38	HIT2	Mouse IgG1, κ	303506
D7	CD39	A1	Mouse IgG1, κ	328208
D8	CD40	HB14	Mouse IgG1, κ	313006
D9	CD41	HIP8	Mouse IgG1, κ	303706
D10	CD42b	HIP1	Mouse IgG1, κ	303906
D11	CD43	CD43-10G7	Mouse IgG1, κ	343204
D12	CD44	BJ18	Mouse IgG1, κ	338808
E1	CD45	HI30	Mouse IgG1, κ	304008
E2	CD45RA	HI100	Mouse IgG2b, κ	304108
E3	CD45RB	MEM-55	Mouse IgG2b, κ	310204
E4	CD45RO	UCHL1	Mouse IgG2a, κ	304206
E5	CD46	TRA-2-10	Mouse IgG1	352402
E6	CD47	CC2C6	Mouse IgG1, κ	323108

Plate 1

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
E7	CD48	BJ40	Mouse IgG1, κ	336708
E8	CD49a	TS2/7	Mouse IgG1, κ	328304
E9	CD49c	ASC-1	Mouse IgG1, κ	343804
E10	CD49d	9F10	Mouse IgG1, κ	304304
E11	CD49e	NKI-SAM-1	Mouse IgG2b, κ	328010
E12	CD49f	GoH3	Rat IgG2a, κ	313612
F1	CD50 (ICAM-3)	CBR-IC3/1	Mouse IgG1, κ	330006
F2	CD51	NKI-M9	Mouse IgG2a, κ	327910
F3	CD51/61	23C6	Mouse IgG1, κ	304406
F4	CD52	HI186	Mouse IgG2b, κ	316006
F5	CD53	HI29	Mouse IgG1, κ	325406
F6	CD54	HA58	Mouse IgG1, κ	353106
F7	CD55	JS11	Mouse IgG1, κ	311308
F8	CD56 (NCAM)	HCD56	Mouse IgG1, κ	318306
F9	CD57	HCD57	Mouse IgM, κ	322312
F10	CD58	TS2/9	Mouse IgG1, κ	330905
F11	CD59	p282 (H19)	Mouse IgG2a, κ	304708
F12	CD61	VI-PL2	Mouse IgG1, κ	336406
G1	CD62E	HAE-1f	Mouse IgG1, κ	336008
G2	CD62L	DREG-56	Mouse IgG1, κ	304806
G3	CD62P (P-Selectin)	AK4	Mouse IgG1, κ	304906
G4	CD63	H5C6	Mouse IgG1, κ	353004
G5	CD64	10.1	Mouse IgG1, κ	305008
G6	CD66a/c/e	ASL-32	Mouse IgG2b, κ	342304
G7	CD66b	G10F5	Mouse IgM, κ	305106
G8	CD69	FN50	Mouse IgG1, κ	310906
G9	CD70	113-16	Mouse IgG1, κ	355104
G10	CD71	CY1G4	Mouse IgG2a, κ	334106

Plate 1

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
G11	CD73	AD2	Mouse IgG1, κ	344004
G12	CD74	LN2	Mouse IgG1, κ	326808
H1	CD79b	CB3-1	Mouse IgG1, κ	341404
H2	CD80	2D10	Mouse IgG1, κ	305208
H3	CD81	5A6	Mouse IgG1, κ	349506
H4	CD82	ASL-24	Mouse IgG1, κ	342104
H5	CD83	HB15e	Mouse IgG1, κ	305308
H6	CD84	CD84.1.21	Mouse IgG2a, κ	326008
H7	CD85a (ILT5)	MKT5.1	Rat IgG2a, κ	337704
H8	CD85d (ILT4)	42D1	Rat IgG2a, κ	338706
H9	CD85g (ILT7)	17G10.2	Mouse IgG1, κ	326408
H10	CD85h (ILT1)	24	Mouse IgG2b, κ	337904
H11	CD85j (ILT2)	GHI/75	Mouse IgG2b, κ	333708
H12	CD85k (ILT3)	ZM4.1	Mouse IgG1, κ	333008

Plate 2

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
A1	Blank			
A2	CD86	IT2.2	Mouse IgG2b, κ	305406
A3	CD87	VIM5	Mouse IgG1, κ	336906
A4	CD88	S5/1	Mouse IgG2a, κ	344304
A5	CD89	A59	Mouse IgG1, κ	354104
A6	CD90 (Thy1)	5E10	Mouse IgG1, κ	328110
A7	CD93	VIMD2	Mouse IgG1, κ	336108
A8	CD94	DX22	Mouse IgG1, κ	305506
A9	CD95	DX2	Mouse IgG1, κ	305608
A10	CD96	NK92.39	Mouse IgG1, κ	338406
A11	CD97	VIM3b	Mouse IgG1, κ	336308
A12	CD99	HCD99	Mouse IgG2a, κ	318008
B1	CD100	A8	Mouse IgG1, κ	328408
B2	CD101 (BB27)	BB27	Mouse IgG1, κ	331006
B3	CD102	CBR-IC2/2	Mouse IgG2a, κ	328506
B4	CD103	Ber-ACT8	Mouse IgG1, κ	350206
B5	CD104	58XB4	Mouse IgG2a, κ	327808
B6	CD105	43A3	Mouse IgG1, κ	323206
B7	CD106	STA	Mouse IgG1, κ	305806
B8	CD107a (LAMP-1)	H4A3	Mouse IgG1, κ	328608
B9	CD108	MEM-150	Mouse IgM, κ	315704
B10	CD109	W7C5	Mouse IgG1, κ	323306
B11	CD111	R1.302	Mouse IgG1, κ	340404
B12	CD112 (Nectin-2)	TX31	Mouse IgG1, κ	337410
C1	CD114	LMM741	Mouse IgG1, κ	346106
C2	CD115	9-4D2-1E4	Rat IgG1, κ	347304
C3	CD116	4H1	Mouse IgG1, κ	305908
C4	CD117 (c-kit)	104D2	Mouse IgG1, κ	313204

Plate 2

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
C5	CD119 (IFN-g R α chain)	GIR-208	Mouse IgG1, κ	308606
C6	CD122	TU27	Mouse IgG1, κ	339006
C7	CD123	6H6	Mouse IgG1, κ	306006
C8	CD124	G077F6	Mouse IgG2a, κ	355004
C9	CD126 (IL-6Rα)	UV4	Mouse IgG1, κ	352804
C10	CD127 (IL-7Rα)	A019D5	Mouse IgG1, κ	351304
C11	CD129 (IL-9 R)	AH9R7	Mouse IgG2b, κ	310404
C12	CD131	1C1	Mouse IgG1, κ	306104
D1	CD132	TUGh4	Rat IgG2b, κ	338606
D2	CD134	Ber-ACT35 (ACT35)	Mouse IgG1, κ	350004
D3	CD135	BV10A4H2	Mouse IgG1, κ	313306
D4	CD137 (4-1BB)	4B4-1	Mouse IgG1, κ	309804
D5	CD137L (4-1BB Ligand)	5F4	Mouse IgG1, κ	311504
D6	CD138	DL-101	Mouse IgG1, κ	352306
D7	CD140a	16A1	Mouse IgG1, κ	323506
D8	CD140b	18A2	Mouse IgG1, κ	323606
D9	CD141	M80	Mouse IgG1, κ	344104
D10	CD143	5-369	Mouse IgG1, κ	344204
D11	CD144	BV9	Mouse IgG2a, κ	348506
D12	CD146	SHM-57	Mouse IgG2a, κ	342004
E1	CD148	A3	Mouse IgG1, κ	328708
E2	CD150 (SLAM)	A12 (7D4)	Mouse IgG1, κ	306308
E3	CD152	L3D10	Mouse IgG1, κ	349906
E4	CD154	24-31	Mouse IgG1, κ	310806
E5	CD155 (PVR)	SKII.4	Mouse IgG1, κ	337610
E6	CD156c (ADAM10)	SHM14	Mouse IgG1, κ	352704
E7	CD158a/h	HP-MA4	Mouse IgG2b, κ	339506

Plate 2

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
E8	CD158b (KIR2DL2/L3, NKAT2)	DX27	Mouse IgG2a, κ	312606
E9	CD158d	mAb 33 (33)	Mouse IgG1, κ	347006
E10	CD158e1 (KIR3DL1, NKB1)	DX9	Mouse IgG1, κ	312708
E11	CD158f	UP-R1	Mouse IgG1, κ	341304
E12	CD161	HP-3G10	Mouse IgG1, κ	339904
F1	CD162	KPL-1	Mouse IgG1, κ	328806
F2	CD163	GHI/61	Mouse IgG1, κ	333606
F3	CD164	67D2	Mouse IgG1, κ	324808
F4	CD165	SN2 (N6-D11)	Mouse IgG1, κ	329010
F5	CD166	3A6	Mouse IgG1, κ	343904
F6	CD167a (DDR1)	51D6	Mouse IgG3, κ	334006
F7	CD169	7-239	Mouse IgG1, κ	346004
F8	CD170 (Siglec-5)	1A5	Mouse IgG1, κ	352004
F9	CD172a (SIRPa)	SE5A5	Mouse IgG1, κ	323806
F10	CD172b (SIRPb)	B4B6	Mouse IgG1, κ	323906
F11	CD172g (SIRPg)	LSB2.20	Mouse IgG1, κ	336606
F12	CD178 (Fas-L)	NOK-1	Mouse IgG1, κ	306407
G1	CD179a	HSL96	Mouse IgG1, κ	347404
G2	CD179b	HSL11	Mouse IgG1, κ	349804
G3	CD180 (RP105)	MHR73-11	Mouse IgG1, κ	312906
G4	CD181 (CXCR1)	8F1/CXCR1	Mouse IgG2b, κ	320608
G5	CD182 (CXCR2)	5E8/CXCR2	Mouse IgG1, κ	320706
G6	CD183	G025H7	Mouse IgG1, κ	353706
G7	CD184 (CXCR4)	12G5	Mouse IgG2a, κ	306506
G8	CD193 (CCR3)	5E8	Mouse IgG2b, κ	310706
G9	CD195 (CCR5)	T21/8	Mouse IgG1, κ	321406
G10	CD196	G034E3	Mouse IgG2b, κ	353410

Plate 2

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
G11	CD197 (CCR7)	G043H7	Mouse IgG2a, κ	353204
G12	CD200 (OX2)	OX-104	Mouse IgG1, κ	329206
H1	CD200 R	OX-108	Mouse IgG1, κ	329306
H2	CD201 (EPCR)	RCR-401	Rat IgG1, κ	351904
H3	CD202b (Tie2/Tek)	33.1 (Ab33)	Mouse IgG1, κ	334206
H4	CD203c (E-NPP3)	NP4D6	Mouse IgG1, κ	324606
H5	CD205 (DEC-205)	HD30	Mouse IgG1, κ	342204
H6	CD206 (MMR)	15-2	Mouse IgG1, κ	321106
H7	CD207 (Langerin)	10E2	Mouse IgG1, κ	352204
H8	CD209 (DC-SIGN)	9E9A8	Mouse IgG2a, κ	330106
H9	CD210 (IL-10 R)	3F9	Rat IgG2a, κ	308804
H10	CD213a2	SHM38	Mouse IgG1, κ	354404
H11	CD215 (IL-15Rα)	JM7A4	Mouse IgG2b, κ	330208
H12	CD218a (IL-18Rα)	H44	Mouse IgG1, κ	313808

Plate 3

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
A1	Blank			
A2	CD220	B6.220	Mouse IgG2b, κ	352604
A3	CD221 (IGF-1R)	1H7/CD221	Mouse IgG1, κ	351806
A4	CD226 (DNAM-1)	11A8	Mouse IgG1, κ	338306
A5	CD229 (Ly-9)	HLy-9.1.25	Mouse IgG1, κ	326108
A6	CD231 (TALLA)	SN1a (M3-3D9)	Mouse IgG1, κ	329406
A7	CD235ab	HIR2	Mouse IgG2b, κ	306604
A8	CD243	UIC2	Mouse IgG2a, κ	348606
A9	CD244 (2B4)	C1.7	Mouse IgG1, κ	329508
A10	CD245 (p220/240)	DY12	Mouse IgG1, κ	334404
A11	CD252 (OX40L)	11C3.1	Mouse IgG1, κ	326308
A12	CD253 (Trail)	RIK-2	Mouse IgG1, κ	308206
B1	CD254	MIH24	Mouse IgG1, κ	347504
B2	CD255 (TWEAK)	CARL-1	Mouse IgG3, κ	308305
B3	CD257 (BAFF, BLYS)	T7-241	Mouse IgG1, κ	318606
B4	CD258 (LIGHT)	T5-39	Mouse IgG2a, κ	318706
B5	CD261 (DR4, TRAIL-R1)	DJR1	Mouse IgG1, κ	307206
B6	CD262 (DR5, TRAIL-R2)	DJR2-4 (7-8)	Mouse IgG1, κ	307406
B7	CD263 (DcR1, TRAIL-R3)	DJR3	Mouse IgG1, κ	307006
B8	CD266 (Fn14, TWEAK Receptor)	ITEM-1	Mouse IgG1, κ	314004
B9	CD267 (TACI)	1A1	Rat IgG2a, κ	311906
B10	CD268 (BAFF-R, BAFFR)	11C1	Mouse IgG1, κ	316906
B11	CD270 (HVEM)	122	Mouse IgG1, κ	318806
B12	CD271	ME20.4	Mouse IgG1, κ	345106
C1	CD273 (B7-DC, PD-L2)	24F.10C12	Mouse IgG2a, κ	329606
C2	CD274 (B7-H1, PD-L1)	29E.2A3	Mouse IgG2b, κ	329706

Plate 3

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
C3	CD275 (B7-H2, B7-RP1, ICOSL)	9F.8A4	Mouse IgG1, κ	329806
C4	CD276	MIH42	Mouse IgG1, κ	351004
C5	CD277	BT3.1	Mouse IgG1, κ	342704
C6	CD278 (ICOS)	C398.4A	Arm. Hamster IgG	313508
C7	CD279 (PD-1)	EH12.2H7	Mouse IgG1, κ	329906
C8	CD282 (TLR2)	TL2.1	Mouse IgG2a, κ	309708
C9	CD284 (TLR4)	HTA125	Mouse IgG2a, κ	312806
C10	CD286 (TLR6)	TLR6.127	Mouse IgG1, κ	334708
C11	CD290	3C10C5	Mouse IgG1, κ	354604
C12	CD294	BM16	Rat IgG2a, κ	350106
D1	CD298	LNH-94	Mouse IgG1, κ	341704
D2	CD300e (IREM-2)	UP-H2	Mouse IgG1, κ	339704
D3	CD300F	UP-D2	Mouse IgG1, κ	340604
D4	CD301	H037G3	Mouse IgG2a, κ	354704
D5	CD303	201A	Mouse IgG2a, κ	354204
D6	CD304	12C2	Mouse IgG2a, κ	354504
D7	CD307	509f6	Mouse IgG2a, κ	340304
D8	CD307d (FcRL4)	413D12	Mouse IgG2b, κ	340204
D9	CD314 (NKG2D)	1D11	Mouse IgG1, κ	320806
D10	CD317	RS38E	Mouse IgG1, κ	348406
D11	CD318 (CDCP1)	CUB1	Mouse IgG2b, κ	324006
D12	CD319 (CRACC)	162.1	Mouse IgG2b, κ	331806
E1	CD324 (E-Cadherin)	67A4	Mouse IgG1, κ	324106
E2	CD325	8C11	Mouse IgG1, κ	350805
E3	CD326 (Ep-CAM)	9C4	Mouse IgG2b, κ	324206
E4	CD328 (Siglec-7)	6-434	Mouse IgG1, κ	339204
E5	CD334 (FGFR4)	4FR6D3	Mouse IgG1, κ	324306

Plate 3

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
E6	CD335 (NKp46)	9E2	Mouse IgG1, κ	331908
E7	CD336 (NKp44)	P44-8	Mouse IgG1, κ	325108
E8	CD337 (NKp30)	P30-15	Mouse IgG1, κ	325208
E9	CD338 (ABCG2)	5D3	Mouse IgG2b, κ	332008
E10	CD340 (erbB2/ HER-2)	24D2	Mouse IgG1, κ	324406
E11	CD344 (Frizzled-4)	CH3A4A7	Mouse IgG1, κ	326606
E12	CD351	TX61	Mouse IgG1, κ	137306
F1	CD352 (NTB-A)	NT-7	Mouse IgG1, κ	317208
F2	CD354 (TREM-1)	TREM-26	Mouse IgG1, κ	314906
F3	CD355 (CRTAM)	Cr24.1	Mouse IgG2a, κ	339106
F4	CD357 (GITR)	621	Mouse IgG1, κ	311604
F5	CD360 (IL-21R)	2G1-K12	Mouse IgG1, κ	347806
F6	β2-micro-globulin	2M2	Mouse IgG1, κ	316306
F7	BTLA	MIH26	Mouse IgG2a, κ	344506
F8	C3AR	hC3aRZ8	Mouse IgG2b	345804
F9	C5L2	1D9-M12	Mouse IgG2a, κ	342404
F10	CCR10	6588-5	Arm. hamster IgG	341504
F11	CLEC12A	50C1	Mouse IgG2a, κ	353604
F12	CLEC9A	8F9	Mouse IgG2a, κ	353804
G1	CX3CR1	2A9-1	Rat IgG2b, κ	341604
G2	CXCR7	8F11-M16	Mouse IgG2b, κ	331104
G3	δ-Opioid Receptor	DOR7D2A4	Mouse IgG2b, κ	327206
G4	DLL1	MHD1-314	Mouse IgG1, κ	346404
G5	DLL4	MHD4-46	Mouse IgG1, κ	346506
G6	DR3 (TRAMP)	JD3	Mouse IgG1, κ	307106
G7	EGFR	AY13	Mouse IgG1, κ	352904
G8	erbB3/HER-3	1B4C3	Mouse IgG2a, κ	324706

Plate 3

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
G9	FceRIa	AER-37 (CRA-1)	Mouse IgG2b, κ	334610
G10	FcRL6	2H3	Mouse IgG2b, κ	338104
G11	Galectin-9	9M1-3	Mouse IgG1, κ	348906
G12	GARP (LRRC32)	7B11	Mouse IgG2b, κ	352504
H1	HLA-A,B,C	W6/32	Mouse IgG2a, κ	311406
H2	HLA-A2	BB7.2	Mouse IgG2b, κ	343306
H3	HLA-DQ	HLADQ1	Mouse IgG1, κ	318106
H4	HLA-DR	L243	Mouse IgG2a, κ	307606
H5	HLA-E	3D12	Mouse IgG1, κ	342604
H6	HLA-G	87G	Mouse IgG2a, κ	335906
H7	IFN-g R b chain	2HUB-159	Hamster IgG	308504
H8	Ig light chain k	MHK-49	Mouse IgG1, κ	316508
H9	Ig light chain λ	MHL-38	Mouse IgG2a, κ	316608
H10	IgD	IA6-2	Mouse IgG2a, κ	348204
H11	IgM	MHM-88	Mouse IgG1, κ	314508
H12	IL-28RA	MHLICR2a	Mouse IgG2a, κ	337804

Plate 4

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
A1	Blank			
A2	Integrin α9β1	Y9A2	Mouse IgG1, κ	351606
A3	integrin β5	AST-3T	Mouse IgG2a, κ	345204
A4	integrin β7	FIB504	Rat IgG2a, κ	321204
A5	Jagged 2	MHJ2-523	Mouse IgG1, κ	346904
A6	LAP	TW4-6H10	Mouse IgG1, κ	349704
A7	Lymphotoxin b Receptor (LT-bR)	31G4D8	Mouse IgG2b, κ	322008
A8	Mac-2 (Galectin-3)	Gal397	Mouse IgG1, κ	126705
A9	MAIR-II	TX45	Mouse IgG1, κ	334804
A10	MICA/MICB	6D4	Mouse IgG2a, κ	320906
A11	MSC (W3D5)	W3D5	Mouse IgG2a, κ	327506
A12	MSC (W5C5)	W5C5	Mouse IgG1, κ	327406
B1	MSC (W7C6)	W7C6	Mouse IgG1, κ	327606
B2	MSC and NPC (W4A5)	W4A5	Mouse IgG1, κ	330806
B3	MSCA-1 (MSC, W8B2)	W8B2	Mouse IgG1, κ	327306
B4	NKp80	5D12	Mouse IgG1, κ	346706
B5	Notch 1	MHN1-519	Mouse IgG1, κ	352106
B6	Notch 2	MHN2-25	Mouse IgG2a, κ	348304
B7	Notch 3	MHN3-21	Mouse IgG1, κ	345406
B8	Notch 4	MHN4-2	Mouse IgG1, κ	349004
B9	NPC (57D2)	57D2	Mouse IgG1, κ	327706
B10	Podoplanin	NC-08	Rat IgG2a, λ	337004
B11	Pre-BCR	HSL2	Mouse IgG1, κ	347904
B12	PSMA	LNI-17	Mouse IgG1, κ	342504
C1	Siglec-10	5G6	Mouse IgG1, κ	347604
C2	Siglec-8	7C9	Mouse IgG1, κ	347104
C3	Siglec-9	K8	Mouse IgG1, κ	351504

Plate 4

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
C4	SSEA-1	MC-480	Mouse IgM, κ	125606
C5	SSEA-3	MC-631	Rat IgM, κ	330312
C6	SSEA-4	MC-813-70	Mouse IgG3, κ	330406
C7	SSEA-5	8E11	Mouse IgG1, κ	355204
C8	TCR g/d	B1	Mouse IgG1, κ	331210
C9	TCR Vβ13.2	H132	Mouse IgG1, κ	333108
C10	TCR Vβ23	αHUT7	Mouse IgG1, κ	349406
C11	TCR Vβ8	JR2 (JR.2)	Mouse IgG2b, κ	348104
C12	TCR Vβ9	MKB1	Mouse IgG2b, κ	349204
D1	TCR Vδ2	B6	Mouse IgG1, κ	331408
D2	TCR Vg9	B3	Mouse IgG1, κ	331308
D3	TCR Vα24-Jα18	6B11	Mouse IgG1, κ	342904
D4	TCR Vα7.2	3C10	Mouse IgG1, κ	351706
D5	TCR α/β	IP26	Mouse IgG1, κ	306708
D6	Tim-1	1D12	Mouse IgG1, κ	353904
D7	Tim-3	F38-2E2	Mouse IgG1, κ	345006
D8	Tim-4	9F4	Mouse IgG1, κ	354004
D9	TLT-2	MIH61	Mouse IgG1, κ	351104
D10	TRA-1-60-R	TRA-1-60-R	Mouse IgM, κ	330610
D11	TRA-1-81	TRA-1-81	Mouse IgM, κ	330708
D12	TSLPR (TSLP-R)	1B4	Mouse IgG1, κ	322806
E1	Ms IgG1, κ ITCL	MOPC-21	Mouse IgG1, κ	400112
E2	Ms IgG2a, κ ITCL	MOPC-173	Mouse IgG2a, κ	400212
E3	Ms IgG2b, κ ITCL	MPC-11	Mouse IgG2b, κ	400314
E4	Ms IgG3, κ ITCL	MG3-35	Mouse IgG3, κ	401320
E5	Ms IgM, κ ITCL	MM-30	Mouse IgM, κ	401609
E6	Rat IgG1, κ ITCL	RTK2071	Rat IgG1, κ	400408

Plate 4

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
E7	Rat IgG2a, κ ITCL	RTK2758	Rat IgG2a, κ	400508
E8	Rat IgG2b, κ ITCL	RTK4530	Rat IgG2b, κ	400636
E9	Rat IgM, κ ITCL	RTK2118	Rat IgM, κ	400808
E10	AH IgG, ITCL	HTK888	Arm. Hamster IgG	400907
E11	Blank			
E12	Blank			
F1	Blank			
F2	Blank			
F3	Blank			
F4	Blank			
F5	Blank			
F6	Blank			
F7	Blank			
F8	Blank			
F9	Blank			
F10	Blank			
F11	Blank			
F12	Blank			
G1	Blank			
G2	Blank			
G3	Blank			
G4	Blank			
G5	Blank			
G6	Blank			
G7	Blank			
G8	Blank			
G9	Blank			
G10	Blank			
G11	Blank			
G12	Blank			
H1	Blank			
H2	Blank			
H3	Blank			
H4	Blank			
H5	Blank			
H6	Blank			
H7	Blank			
H8	Blank			
H9	Blank			

Plate 4

Well ID	Specificity	Clone	Isotype	BioLegend Cat. No.
H10	Blank			
H11	Blank			
H12	Blank			

Notes

Tel: 858-768-5800

Notes

LEGENDScreen™ Kits are manufactured by **BioLegend Inc.**
9727 Pacific Heights Blvd.
San Diego, CA 92121
Tel: 1.858.768.5800
Tel: US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343)
Fax: 1.877.455.9587
Email: info@biolegend.com
biolegend.com

For a complete list of world-wide BioLegend offices and distributors, please visit our website at: biolegend.com