How to Flow Sort Adherent Human and Mouse Cells Using Cell Surface Markers

Reagents and Buffers

1) 1x Dulbecco's PBS (Ca^{++}/Mg^{++} Free) (Thermo Fisher Cat# SH3002802).
2) 1.0M HEPES (Thermo Fisher Cat# 15-630-080; Cat# ICN1688449; or Cat# AAJ60712AK).
3) 0.5M EDTA pH 8.0 (Thermo Fisher Cat# BP2482-500).
4) Bovine serum albumin (BSA) (Millipore Sigma Cat# A9418 or Cat# A3311), or FBS [dialyzed against 1x Dulbecco's PBS (Ca^{++}/Mg^{++} Free)].
5) 500x Pen/Strep antibiotics stock solution.
6) Fluorescence-labeled antibodies against **cell surface markers**:

   Human cell-specific markers include HLA-ABC or HLA-A.
   BD Pharmingen™ **FITC Mouse Anti-Human HLA-ABC Clone G46-2.6 (RUO)** (*BD Biosciences Catalog No: 555552; Fisher Cat# **BDB555552** for 100 tests; stored @ 4°C*)

   Mouse cell-specific markers include MHC Class I molecules H-2Db or H-2Kd/H-2Dd (see the attached **Mouse Haplotype Table**)
   BD Pharmingen™ **Alexa Fluor™ 488 Mouse Anti-Mouse H-2Kd/H-2Dd Clone 34-1-2S (RUO)** (*BD Biosciences Catalog No: 567696; Fisher Cat# **BDB567696***)
   BD Pharmingen™ FITC Mouse Anti-Mouse H-2D[b] Clone KH95 (RUO) (*BD Biosciences Catalog No: 562000; Fisher Cat# **BDB562000***)

   Mouse MHC Class II (I-A/I-E) antibodies (primarily immune cells and epithelial cells):
   BD Pharmingen™ Alexa Fluor® 488 Rat Anti-Mouse I-A/I-E Clone M5/114.15.2 (aka M5/114) (RUO) (*BD Biosciences Catalog No: 562352; Fisher Cat# BDB562352*)
   BD Pharmingen™ PE Rat Anti-Mouse I-A/I-E Clone M5/114.15.2 (aka. M5/114) (RUO) (*BD Biosciences Catalog No: 562010; Fisher Cat# **BDB562010***)
   BD Pharmingen™ FITC Rat Anti-Mouse I-A/I-E Clone 2G9 (RUO) (*BD Biosciences Catalog No: 562009; Fisher Cat# BDB562009*)

7) BD Pharmingen™ Stain Buffer (BSA) (*BD Cat# 554657; Thermo Fisher Cat# BDB554657*) or BD Pharmingen™ Stain Buffer (FBS) (*BD Cat# 554656; Thermo Fisher Cat# BDB554656*)

Cell Detachment Buffer (CDB)

1) 1x Dulbecco's PBS (Ca^{++}/Mg^{++} Free)
2) 25 mM HEPES
3) 5mM EDTA (cell type-dependent)
4) 1x Pen/Strep antibiotics

To make 100mL, add **2.5mL** of 1.0M HEPES, **1.0mL** of 0.5M EDTA pH 8.0, **200µL** of 500x Pen/Strep antibiotics stock, to **96.3mL** of 1x Dulbecco's PBS (Ca^{++}/Mg^{++} Free).

Cell Sorting Buffer (CSB)

1) 1x Dulbecco's PBS (Ca^{++}/Mg^{++} Free)
2) 25 mM HEPES
3) 1% FBS (For some cell types, 1% BSA is better than 1% FBS)
4) 2.0mM EDTA (may vary 1~5mM, depending on cell types)
5) 1x Pen/Strep antibiotics

To make 100mL, add **2.5mL** of 1.0M HEPES, **1.0mL** FBS, **400µL** of 0.5M EDTA pH 8.0, **200µL** of 500x Pen/Strep antibiotics stock, to **95.9mL** of 1x Dulbecco's PBS (Ca^{++}/Mg^{++} Free).

Cell Collection Medium (CCM)

1) Use the **complete DMEM** or cell-specific complete culture medium for collecting the sorted cells. It is important to make sure antibiotics (e.g., Pen/Strep) are added to the medium.
2) For some primary cells with low viability and/or low positivity, you can use 100% FBS (with antibiotics) as cell collection medium.

Cell Detachment and Isolation

1) First, book a Cell Sorter at the UChicago CAT Facility's iLab online scheduler (***NOTE**: You have to be trained by the core facility, and need to use your CNet ID/UChicago email account for login*).
2) Culture adherent cells in 100mm culture dishes or T-25 flasks to 80 to 90% confluence in complete medium.
3) Remove complete medium, wash the cells with 1x Dulbecco's PBS (Ca^{++}/Mg^{++} Free) x 1-2 times.
4) Add Cell Detachment Buffer (CDB) to cell culture, monitor cell detachment closely (***NOTE**: Most adherent cells come off in about 5-15min; Check cell detachment under microscope every 3-5min*).
5) Collect the detached cells in Cell Sorting Buffer (CSB) and spin down the cells at 300xg for 10min.

Direct Immunofluorescent Staining and Cell Sorting

1) Prepare single-cell suspensions from adherent cell cultures as described above.
2) Wash the cells twice in cold **Pharmingen Stain Buffer (BSA)** and pellet the cells by centrifugation (e.g., 300 x g at 4°C). Resuspend the cell pellet with cold **Pharmingen Stain Buffer (BSA)** to a final concentration of 2 x 10e7 cells/ml.
3) Distribute 50µl aliquots of the cell suspension (~10e6 cells) to either tubes or the round-bottomed wells of microwell plates.
4) Dilute fluorescent antibodies to their predetermined optimal concentrations in **Pharmingen Stain Buffer** and add small aliquots (e.g., 10 µl) of the diluted antibodies to the tubes or microwells that contain the target cell suspensions. **For the BD Pharmingen™ FITC Mouse Anti-Human HLA-ABC Clone G46-2.6 (RUO) kit, each test contains 20µl FITC-labeled antibody.** However, pilot experiments are needed to determine the specificity and sensitivity of the antibody using mouse cells as negative controls. The antibody may need to be further diluted**.**
5) Incubate for **20 minutes on ice protected from light**. Staining time may be increased (≥ 45 min) depending on the avidity of the fluorescent antibody.
6) Wash the cells two times with either 200 µl (for microwell plates) or 1 ml (for tubes) volumes of **Pharmingen Stain Buffer** to remove unbound antibodies. Centrifuge cells as 300 x g for 5 min. After each centrifugation, carefully aspirate (for microwell plates or tubes) or invert and blot away (for tubes) supernatants from cell pellets.
7) Resuspend the cell pellet in either 200 µl (for microwell plates) or 0.5 ml (for tubes) volumes of **Pharmingen Stain Buffer** or **Cell Sorting Buffer**. Transfer stained cells from microwell plates to the appropriate tubes for flow cytometric analysis (adjust final volume to ~0.5 ml). **For cell sorting, cell concentration should be adjusted to 3~5 million cells/mL**.
8) Sorted cells are collected in **Cell Collection Medium** (containing antibiotics); and propagate the cells in 37°C and 5% CO_2 incubator.

NOTES:

1) Analyze stained cell samples either by flow cytometry or by fluorescence microscopy as soon as possible (e.g., ≤ 4 hours) after staining. If analysis must be delayed, then the stained cells can be fixed with buffered paraformaldehyde (e.g., Cytofix Buffer; Cat. No. 554657) and stored at 4°C (protected from

light). The fixed cells should be analyzed as soon as possible (e.g., up to one week after staining and fixation).

2). Pharmingen Stain Buffer (BSA) can similarly be used for the indirect immunofluorescent staining of cells. In this case, repeat steps 4 and 5 when using either unlabeled or biotinylated primary antibodies.

3). Pharmingen Stain Buffer (BSA) can also be used for the immunofluorescent staining of surface antigens expressed by cells that are destined to be fixed and immunofluorescently stained for intracellular antigens such as cytokines (for details see reference #6 or the online protocols at our web site at www.bdbiosciences.com/pharmingen/protocols/). Cells stained for intracellular cytokines can be resuspended and maintained (i.e., at 4°C, protected from light) in Pharmingen Stain Buffer (BSA) prior to analysis by either flow cytometry or fluorescence microscopy.

CELL SORTING TIPS

- **Centrifugation**: Use minimal speed to sediment cells. A good starting point for most preparations is 300xg for 10 minutes.
- **Vortexing**: Avoid vigorous vortexing.
- **Pelleting**: Do not generate a dry pellet at any time during processing.
- **Air Bubbles**: Avoid introducing air bubbles. Surface tension forces can kill cells.
- **Temperature**: Keep samples on ice, unless otherwise required by a specific protocol. Slowing intracellular metabolism helps cells survive longer outside the incubator.
- **For sticky cells**: Try increasing the concentration of EDTA to 5mM and use FBS that has been dialyzed against Ca/Mg++free PBS or 0.5% BSA. Use Ca++/Mg++-free buffers. PBS without Ca/Mg is advised. This helps to reduce cell aggregation. Include 0.1-1% BSA or dialyzed FBS at 1-5%. Use a minimal amount of BSA to decrease autofluorescence and to increase population resolution. Avoid non-dialyzed FBS, as it facilitates cell-cell adhesion by replacing Ca and Mg. Add EDTA at 2-5mM to help prevent cell adhesion.
- **Use of HEPES**: High pressure during sorting compromises buffer capacity. Add 10-25mM HEPES to improve pH stability.
- **Use of DNaseI**: To samples with reduced cell viability, omit EDTA and add 25-50 ug/mL DNAseI with 5mM MgCl2. This digests free DNA released by dead cells.
- **No high cell concentrations**: Avoid keeping cells at unnecessarily high concentration. Keep the cell suspension at 1-10 million/mL during processing, depending on cell type. Counting cells just prior to final resuspension will aid in accurately determining how many cells you are starting with, what volume to resuspend in, and estimate the maximum yield for each sorted population.
- **No phenol red**: Do NOT use sorting buffers that contain phenol red, as this causes an increase in background fluorescence and decreases sensitivity. If using RPMI as your sorting buffer, which is not recommended, make sure there is NO phenol red (highly autofluorescent) and that 25mM of HEPES is added.
- **Not use of culture medium**: Culture media is NOT ideal for sorting for the following reasons: The pH becomes basic under normal atmosphere reducing the cell viability. The calcium chloride in most culture media is incompatible with the phosphate component of the instrument sheath buffer, causing calcium phosphate crystals to form.
- **Avoid losing sorted cells**: Cells sticking to the sides of the collection tube may also affect your cell yield. Pre-coating the collection tubes with fetal calf serum helps prevent this. Sorting is not 100% efficient! There is cell loss before, during, and after the sorting process. The healthier the cells at the beginning, the better the results of the sort will be.
- **Sterility - use of antibiotics.** While we thoroughly sterilize the sample delivery part of the sorter before each sort and sterilize the entire sheath fluid path once every week (sheath fluid goes through an in-line 0.2um filter as well) in our operating environment the potential for airborne contamination, which in our experience is very rare, is possible. We recommend that when culturing sorted cells you add antibiotics which include 50µg/ml gentamycin. You may leave pen-strep etc. in as well. If a long term culture is desired the gentamycin can be discontinued after about a week. We have done many successful sterile sorts where the investigators used no antibiotics following the sort but since your sorted cells are valuable we suggest prudence.
- **Sorting Speed -** Even though all our sorters are considered "high speed", as are all sorters now manufactured, the actual number of cells that can be sorted per second (actually number of input cells per sec) depends on a number of factors. High speed sorters are not high speed because we can put more cell volume through per second. They are high speed because of the characteristics of the electronics and because they can use higher sheath pressure to attain higher droplet rates which permit a higher number of cells per second to be processed. However, we cannot achieve the higher cell throughput rates by pushing a higher volume of sample through per second. To achieve higher cell input rates the cells must be at higher cell concentrations. Not all cells can handle these concentrations and so cannot be sorted as fast. Also larger cells must be sorted using a larger nozzle at lower sheath pressures and, thus, generate fewer drops /sec. The nature of the experiment can also dictate the speed. If sorting rare cells and 'enrich mode' will suffice, then higher speeds may be attainable. If high purity is the goal

then lower speeds must be used.

- **Cell Detachment/Isolation**: You can use ACCUTASE or ACCUMAX (Non-enzymatic cell dissociation buffers) for detaching cells, which are the best options to use. The samples can stay in either buffer throughout your entire sort. Take note that Accutase can alter some surface epitopes and this effect will need to be determined empirically for the epitopes being evaluated. The two products, Accutase and Accumax, are from Innovative Cell Technologies for cell dissociation. Accutase gently detaches confluent cells from plastic ware, does not have to be neutralized like trypsin, and preserves epitopes that can be stained by flow cytometry. Accumax breaks up aggregates in suspension culture and can extend the sort time of a sample. http://www.accutase.com/
- **Recommendations for trypsinized adherent cells**: Adherent cell lines can reaggregate when serum is used to inactivate trypsin. After trypsin treatment, for detaching adherent cells in the plate, Soybean Trypsin Inhibitor is recommended to stop trypsinization instead of using serum. When using trypsin to detach adherent cells, you can use FBS that has been dialyzed against Ca/Mg++ free PBS or increase the EDTA concentration to 5mM or higher (first make sure that your specific cell type is not sensitive to high concentrations of EDTA). Each cell type responds differently to SBTI and the ideal conditions must be determined empirically, but usually between 0.05%-0.25% SBTI in HBSS will work.

UChicago Cytometry (CAT) Core Facility Website

https://voices.uchicago.edu/ucflow/

Features and Availability of Cell Sorters

https://voices.uchicago.edu/ucflow/cell-sorters/

Cell Sorter Feature Comparison

	FACSAriall 4-15	FACSAria III 3-15	FACSAria Fusion 5-18	Miltenyi Tyto	Bigfoot
Number of lasers	4	3	5	3	5
Droplet-based sorter	✓	✓	✓		✓
Sample enclosed in sterile cartridge				✓	
Compensation	✓	✓	✓	✓	✓
Spectral Unmixing					✓
Maximum number of sorted populations (simultaneous)	4	4	4	1*	6
Bulk sorting	✓	✓	✓	✓	✓
Sorting into plates	✓		✓		✓
Human or biohazardous samples			✓	✓	✓
Pressure (psi)	~30-70	~30-70	~30-70	~3	~30-70

*The Tyto can sort more than one population, but this is achieved sequentially instead of simultaneously.

		FACSAria Fusion	Bigfoot
UV	BUV396	X	54 detectors available Can detect pretty much any excitable fluorophore
UV	BUV496	X	
UV	BUV805	X	
405nm	BV421	X	
405nm	BV510	X	
405nm	BV605	X	
405nm	BV650	X	
405nm	BV711	X	
405nm	BV786	X	
488nm	FITC	X	
488nm	PerCP-Cy5.5	X	
561nm	PE	X	
561nm	PE-Dazzle595	X	
561nm	PE-Cy5	X	
561nm	PE-Cy5.5	X	
561nm	PE-Cy7	X	
640nm	APC	X	
640nm	APCR700	X	
640nm	APC-Cy7	X	

5 lasers cell sorters

		FACSAria III	MACSQuant Tyto
Violet	BV421	X	V1
Violet	BV510	X	V2
Violet	BV605	X	
Violet	BV650	X	
Violet	BV711	X	
Violet	BV786	X	
Blue	FITC	X	B1
Blue	PE	X	B2
Blue	PerCP-Cy5.5	X	B3
Blue	PE-Dazzle594	X	B3
Blue	PE-Cy5	X	B3
Blue	Pe-Cy7	X	B4
Red	APC	X	R1
Red	APCR700	X	
Red	APC-Cy7	X	R2

3 lasers cell sorters

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Mouse Haplotype Table

		MHC Class I			MHC Class II		MHC Class Ib				
Mouse Strains	MHC Haplotype	H-2K	H-2D	H-2L	I-A	I-E	Qa-2	Qa-1	CD45 (Ly-5)	Thy-1 (CD90)	NK1.1
101/-	k	k	k	null	k	k					
129/-	b	b	b	null	b	null	a	b	2	2	-
A/J	a	k	d	d	k	k	a	a	2	2	-
A/2G	a	k	d	d	k	k					
AKR/J	k	k	k	null	k	k	b	b	2	1	-
AL/N	a	k	d	d	k	k	a				
AU/SsJ	q	q	q	q	q	null					-
BaLB/cAnN	d	d	d	d	d	d	null			2	-
BALB/cBy	d	d	d	d	d	d	null	b			
BALB/CJ	d	d	d	d	d	d	a	b	2	2	-
BDP/J	p	p	p	p	p	p	b		2		
BUB/BnJ	q	q	q	q	q	null				1	
BXSB/Mp	b	b	b	null	b	null				2	
C3H/Bi	k	k	k	null	k	k	b			2	
C3H/He	k	k	k	null	k	k	b	b	2	2	-
C3HeB/FeJ	k	k	k	null	k	k					
C57BL/6	b	b	b	null	b	null	a	b	2	2	+
C57BL/10	b	b	b	null	b	null	a	b	2	2	
C57BR/cdJ	K2	k	k	k	k	k	b	a	2	2	-
C57L/J	bc	b	b	null	b	null	a	b	2	2	
C58/J	K2	k	k	null	k	k	b	a	2	2	
C.B-17	d	d	d	d	d	d	a	b		2	-
CBA/Ca	k	k	k	null	k	k	b		2	2	
CBA/J	k	k	k	null	k	k	b	b	2	2	-
CBA/N	k	k	k	null	k	k	b		2	2	
CE/J	k	k	k	null	k	k	b		2	2	+
DA/HuSn	qp	q	s		q				1	2	
DBA/1	q	q	q	q	q	null	a		2	2	-
DBA/2	d	d	d	d	d	d	a	b	2	2	-
FVB/N	q	q	q	q	q	null				1	+
GRS/J	dx	d	w3		f	null			2	2	
HRS/J	k	k	k	null	k	k					
I/LnJ	j	j	b	null	j	j				2	
LP/J	bc	b	b	null	b	null			2	2	
MA/MyJ	k	k	k	null	k	k	b			1	+
MRL/Mp	k	k	k	null	k	k				2	-
NOD	g7	d	b	blank	g7	null	a			2	-
NZB/-	d2	d	d	d	d	d	a	a	2	2	+
NZW/-	z	u	z	z	u	u	b				+
P/J	p	p	p	p	p	p	b			1	
PL/J	u	u	d	d	u	u			2	1	
RF/J	k	k	k	null	k	k	b		2	1	
RIII/-	r	r	r	r	r	r	b	c	1	2	
SEC/-	d	d	d	d	d	d				2	+
SJL/J	s2	s	s	s	s	null	a	a	1	2	+
SM/J	v	v	v	v	v	v				2	-
ST/bJ	k	k	k	null	k	k			2	2	+
SWR/J	q2	q	q	q	q	null	a	a	2	2	

MHC Class I Antibodies						
	MHC Class I (H-2Db) Clone 28-14-8	MHC Class I (H-2Kb) Clone AF6-88.5.5.3	MHC Class I (H-2Kd) Clone SF1-1.1.1	MHC Class I (H-2Kd/H-2Dd) Clone 34-1-2S	MHC Class I (H-2Kk) Clone AF3-12.1.3	MHC Class I (H-2Dk) Clone 15-5-5
101/-					+	+
129/-	+	+		+		
A/J	+			+	+	
A/2G	+			+	+	
AKR/J					+	+
AL/N	+			+	+	
AU/SsJ	+			+		
BaLB/cAnN	+		+	+		
BALB/cBy	+		+	+		
BALB/CJ	+		+	+		
BDP/J				+		
BUB/BnJ	+			+		
BXSB/Mp	+	+		+		
C3H/Bi					+	+
C3H/He					+	+
C3HeB/FeJ					+	+
C57BL/6	+	+		+		
C57BL/10	+	+		+		
C57BR/cdJ					+	+
C57L/J	+	+		+		
C58/J					+	+
C.B-17	+		+	+		
CBA/Ca					+	+
CBA/J					+	+
CBA/N					+	+
CE/J					+	+
DA/HuSn				+		
DBA/1	+			+		
DBA/2	+		+	+		
FVB/N	+			+		
GRS/J			+	+		
HRS/J					+	+
I/LnJ	+					
LP/J	+	+		+		
MA/MyJ					+	+
MRL/Mp					+	+
NOD	+		+	+		
NZB/-			+	+		
NZW/-						
P/J				+		
PL/J	+			+		
RF/J					+	+
RIII/-				+		
SEC/-	+		+	+		
SJL/J				+		
SM/J						
ST/bJ					+	+
SWR/J	+			+		

MHC Class II Antibodies				
	MHC Class II (I-A/I-E) Clone M5/114.15.2	MHC Class II (I-Ab) Clone AF6-120.1	MHC Class II (I-Ad) Clone AMS-32.1	MHC Class II (I-Akα) Clone 11-5.2
101/-	+	+		+
129/-	+	+		
A/J	+	+		
A/2G	+	+		
AKR/J	+	+		+
AL/N	+	+		
AU/SsJ	+			
BaLB/cAnN	+		+	
BALB/cBy	+		+	
BALB/CJ	+		+	
BDP/J	+			
BUB/BnJ	+			
BXSB/Mp	+	+		
C3H/Bi	+	+		+
C3H/He	+	+		+
C3HeB/FeJ	+	+		+
C57BL/6	+	+		
C57BL/10	+	+		
C57BR/cdJ	+	+		+
C57L/J	+	+		
C58/J	+	+		+
C.B-17	+		+	
CBA/Ca	+	+		+
CBA/J	+	+		+
CBA/N	+	+		+
CE/J	+	+		+
DA/HuSn	+			
DBA/1	+			
DBA/2	+		+	
FVB/N	+			
GRS/J			+	
HRS/J	+	+		+
I/LnJ				
LP/J	+			
MA/MyJ	+	+		+
MRL/Mp	+	+		+
NOD			+	
NZB/-	+		+	
NZW/-		+		
P/J	+			
PL/J		+		
RF/J	+	+		+
RIII/-				+
SEC/-	+		+	
SJL/J				
SM/J			+	
ST/bJ	+	+		+
SWR/J	+			

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FC03114 Mouse Haplotype Table

Technical Data Sheet

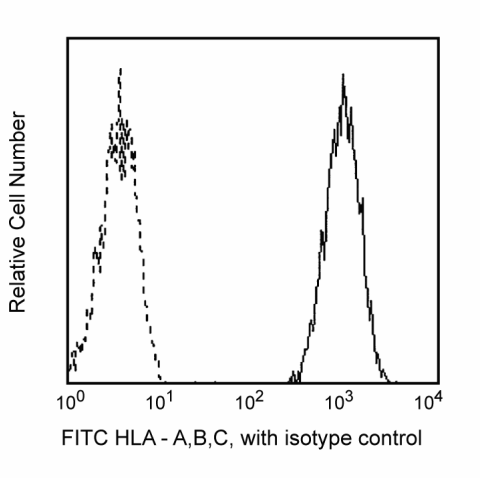
FITC Mouse Anti-Human HLA-ABC

Product Information

Material Number:	**555552**
Alternate Name:	Major histocompatibility complex, class I, A,B,C; HLA class I A,B,C
Size:	100 Tests
Vol. per Test:	20 µl
Clone:	G46-2.6
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The Human Leukocyte Antigen (HLA) complex is the human version of the MHC, helping the immune system distinguish the body's own proteins versus those from foreign invaders, such as viruses. Humans have three main MHC class I genes, known as HLA-A, HLA-B and HLA-C. Major histocompatibility complex (MHC) class I molecules, which are widely found on the surface of nucleated cells, function by binding peptides and displaying them on the cell surface to cytotoxic T-cells. Intracellular degradation of cytosolic proteins by the proteasome generates many of the peptides that load MHC class I molecules. MHC class I may also serve as an inhibitory ligand for natural killer (NK) cell receptors (KIR, Killer Immunoglobulin-like Receptors), which viruses may modulate expression levels for to evade immune detection. The G46-2.6 monoclonal antibody binds to a monomorphic epitope on the alpha chain of HLA-A, HLA-B and HLA-C.



Flow cytometric analysis of HLA-ABC expression on human peripheral blood lymphocytes. *Whole blood was stained with either FITC Mouse Anti-Human HLA-ABC (Cat. No.555552/560965/557348; solid line histogram) or FITC Mouse IgG1, κ Isotype Control (Cat. No. 555748; dashed line histogram). Erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). Fluorescence histograms depicting HLA-ABC (or Ig isotype control) expression were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed on a BD FACScan™ system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Suggested Companion Products

Catalog Number	Name	Size	Clone
555748	FITC Mouse IgG1, κ Isotype Control	100 Tests	MOPC-21
560965	FITC Mouse Anti-Human HLA-ABC	25 Tests	G46-2.6
557348	FITC Mouse Anti-Human HLA-ABC	50 Tests	G46-2.6
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Barclay NA, Brown MH, Birkeland ML, et al, ed. *The Leukocyte Antigen FactsBook.* San Diego, CA: Academic Press; 1997(Biology)

Technical Data Sheet

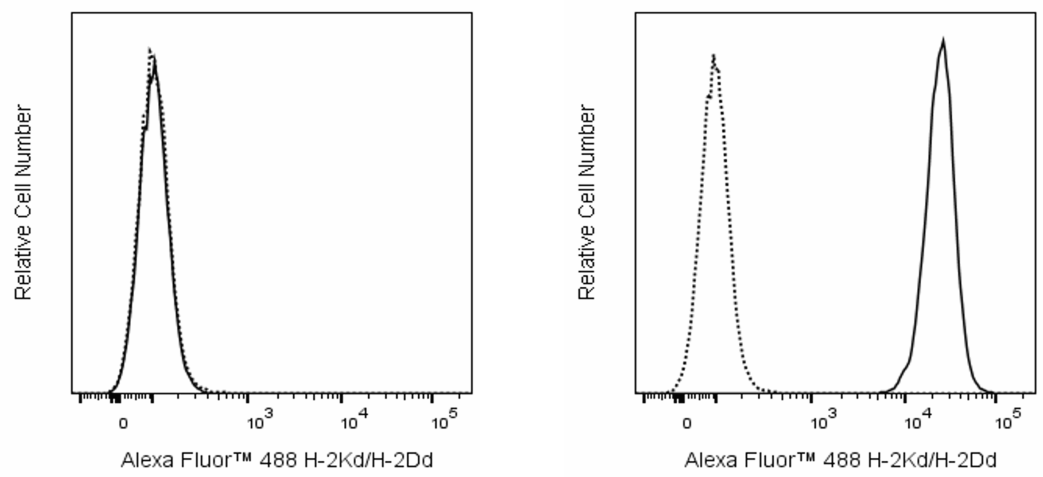
Alexa Fluor™ 488 Mouse Anti-Mouse H-2Kd/H-2Dd

Product Information

Material Number:	**567696**
Alternate Name:	H2-D1, H-2D; H2-K1, H-2K, H2-K
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	34-1-2S
Immunogen:	C57BL/6 × DBA/2 (BDF1) splenocytes
Isotype:	Mouse (C3H) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 34-1-2S monoclonal antibody specifically recognizes the mouse histocompatibility 2 (H-2) alloantigens, H-2Kd and H-2Dd, that are variably expressed on most nucleated cells. These MHC class I antigens are heterodimers comprised of a polymorphic alpha heavy chains (~44 kDa type I transmembrane glycoprotein encoded by the H-2 gene complex) that are noncovalently associated with invariant β2-microglobulin (~11 kDa beta light chain encoded by *B2m*). H-2Kd and H-2Dd are involved in the positive and negative selection of CD8+ T cells in the thymus as well as MHC-restricted antigen presentation to CD8+ αβ T cells in the periphery. H-2Kd and H-2Dd molecules can also serve a ligands for activating or inhibitory receptors, such as, those encoded by the Ly49 gene family which are variably expressed on subsets of natural killer (NK) cells and T cells. The 34-1-2S antibody cross-reacts with H-2K MHC class I alloantigens of the *b*, *s*, *r*, *q*, or *p* haplotypes.



Flow cytometric analysis of H-2Kd/H-2Dd expression on BALB/c mouse splenocytes. *Splenic leucocytes from a C3H (H-2k haplotype; Left Plot) or a BALB/c (H-2d haplotype; Right Plot) mouse were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with Alexa Fluor™ 488 Mouse IgG2a, κ Isotype Control (Cat. No. 565358; dashed line histograms) or with Alexa Fluor™ 488 Mouse Anti-Mouse H-2Kd/H-2Dd antibody (Cat. No. 567696; solid line histograms) at 0.25 µg/test. BD Via-Probe™ Cell Viability 7-AAD Solution (Cat. No. 555815/555816) was added to cells right before analysis. The fluorescence histograms showing H-2Kd/H-2Dd expression (or Ig Isotype control staining) were derived from gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ X-20 Cell Analyzer System and FlowJo™ software. Data shown on this Technical Data Sheet are not lot specific.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to the dye under optimum conditions and unreacted dye was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

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Recommended Assay Procedure:

BD® CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to BD® CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and BD® CompBeads to ensure that BD® CompBeads are appropriate for your specific cellular application.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554657	Stain Buffer (BSA)	500 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
555899	Lysing Buffer	100 mL	(none)
565358	Alexa Fluor® 488 Mouse IgG2a, κ Isotype Control	50 µg	G155-178
555815	Cell Viability Solution	500 Tests	(none)
555816	Cell Viability Solution	100 Tests	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2

Product Notices

1. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.
2. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. An isotype control should be used at the same concentration as the antibody of interest.
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8. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
9. Alexa Fluor™ is a trademark of Life Technologies Corporation.

References

Boudreau JE, Hsu KC. Natural Killer Cell Education and the Response to Infection and Cancer Therapy : Stay Tuned. *Trends Immunol.* 2018; 39(3):222-239. (Methodology)

Brennan J, Mager D, Jefferies W, Takei F. Expression of different members of the Ly-49 gene family defines distinct natural killer cell subsets and cell adhesion properties. *J Exp Med.* 1994; 180(6):2287-2295. (Clone-specific: Blocking)

Lenz A, Heufler C, Rammensee HG, et al. Murine epidermal Langerhans cells express significant amounts of class I major histocompatibility complex antigens. *Proc Natl Acad Sci U S A.* 1989; 86(19):7527-31. (Clone-specific: Flow cytometry, Immunofluorescence)

Ozato K, Mayer NM, Sachs DH. Monoclonal antibodies to mouse major histocompatibility complex antigens IV. A series of hybridoma clones producing anti-H-2d antibodies and an examination of expression of H-2d antigens on the surface of these cells. *Transplantation.* 1982; 34(3):113-120. (Immunogen: Cytotoxicity, Radioimmunoassay)

Pereira RA, Simmons A. Cell surface expression of H2 antigens on primary sensory neurons in response to acute but not latent herpes simplex virus infection in vivo. *J Virol.* 1999; 73(8):6484-9. (Clone-specific: Flow cytometry)

Schenkel AR, Kingry LC, Slayden RA. The ly49 gene family. A brief guide to the nomenclature, genetics, and role in intracellular infection. *Front Immunol.* 2013; 4:90. (Biology)

Technical Data Sheet

FITC Mouse Anti-Mouse H-2D[b]

Product Information

Material Number:	**562000**
Alternate Name:	Histocompatibility-2Db; H-2Db
Size:	50 µg
Concentration:	0.5 mg/ml
Clone:	KH95
Immunogen:	C57BL/10 mouse skin graft and splenocytes
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The KH95 antibody specifically recognizes the H-2D[b] MHC class I alloantigen. Reactivity with other haplotypes (e.g., *a, d, f, k, n, p, q, r, s, u, v*) has not been observed.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
559532	FITC Mouse IgG2b, κ Isotype Control	0.25 mg	MPC-11
553573	FITC Mouse Anti-Mouse H-2D[b]	0.5 mg	KH95
554657	Stain Buffer (BSA)	500 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Hasenkrug KJ, Cory JM, Stimpfling JH. Monoclonal antibodies defining mouse tissue antigens encoded by the H-2 region. *Immunogenetics.* 1987; 25(2):136-139. (Immunogen)

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Technical Data Sheet

PE Rat Anti-Mouse I-A/I-E

Product Information

Material Number:	**562010**
Alternate Name:	H-2I; I-Ab, I-Ad, I-Aq, I-Ed, and I-Ek MHC class II alloAgs; Ia Ag; M5/114; MHC II
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	M5/114.15.2 (also known as M5/114)
Immunogen:	Activated C57BL/6 Mouse Spleen Cells
Isotype:	Rat (BN x LEW) IgG2b, κ
Reactivity:	QC Testing: Mouse
RRID:	AB_396546
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The M5/114.15.2 monoclonal antibody recognizes a polymorphic determinant shared by the I-A[b], I-A[d], I-A[q], I-E[d], and I-E[k] (but not I-A[f], I-A[k], or I-A[s]) MHC class II alloantigens that can be expressed by B cells, dendritic cells, monocytes, macrophages and activated T cells. It also reacts with cells from mice of the H-2[p] and H-2[r] haplotypes, and it is non-reactive with cells from NOD (H-2[g7]) mice. Flow cytometric analysis indicates that the M5/114.15.2 and 2G9 monoclonal antibodies have comparable reactivity on cells from mice with I-A[b], I-A[d], I-A[g7], I-A[q], I-E[d], and I-E[k] alloantigens.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Bhattacharya A, Dorf ME, Springer TA. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. *J Immunol.* 1981; 127(6):2488-2495. (Immunogen)
Hattori M, Buse JB, Jackson RA, et al. The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex. *Science.* 1986; 231(4739):733-735. (Clone-specific)

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