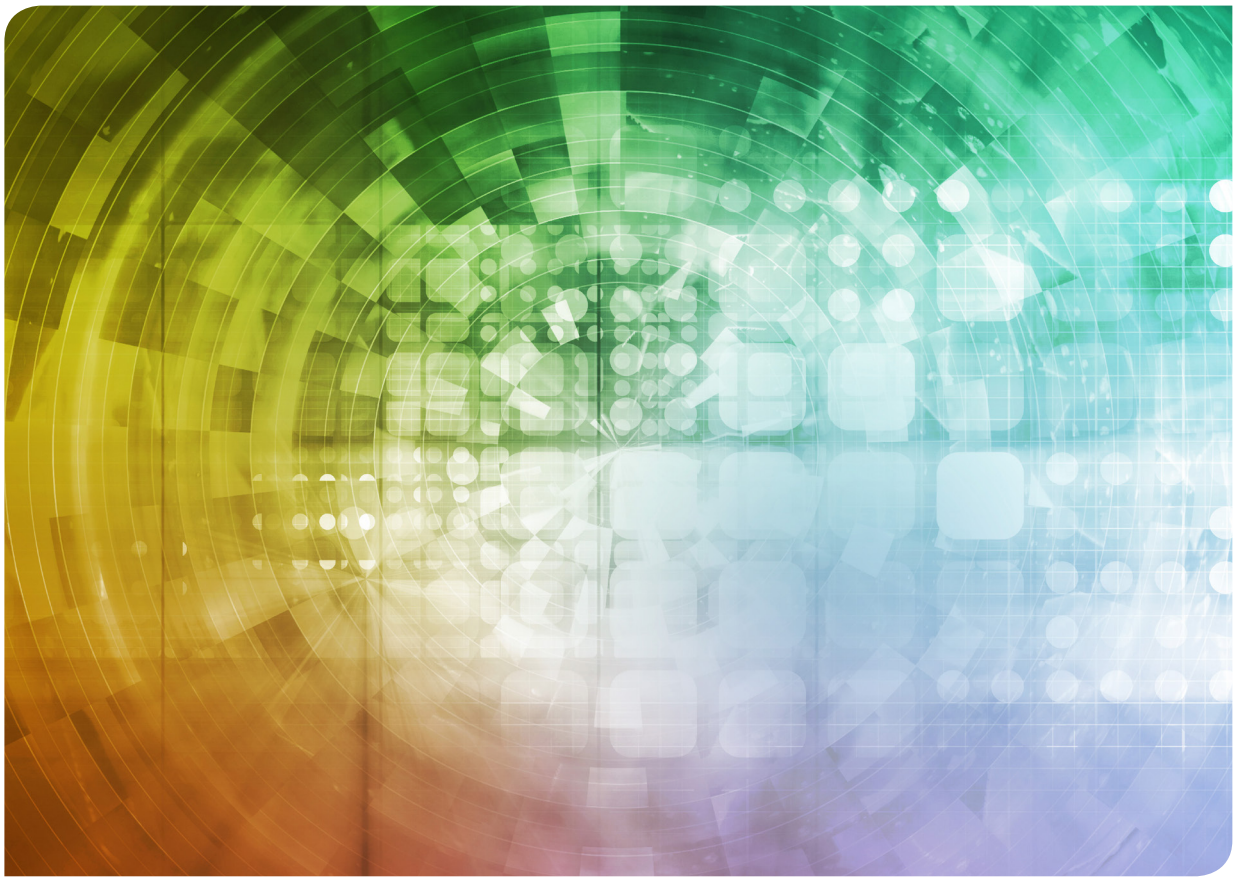


Isotype Controls

Determine Specific Binding

What are Isotype Controls and When to Use them

Unwanted background cell surface staining in flow cytometry can be a problem, especially when detecting novel or rare populations and when building panels containing multiple fluorophores. The level of background staining can be determined using isotype controls.

An isotype control is an antibody raised against an antigen not present on the cell type being analyzed (e.g. KLH or DNP) and has been specifically developed to determine the level of background staining.

An isotype control will:

- Determine the non-specific binding of antibody to Fc receptors found on monocytes, macrophages, dendritic and B cells
- Ensure the observed staining is due to specific binding rather than an artefact
- Reveal other non-specific binding of the antibody or fluorophores to cellular components (e.g. RPE and FITC, Takizawa et al. 1993, Hulspas et al. 2006)

How an isotype control can help determine background staining

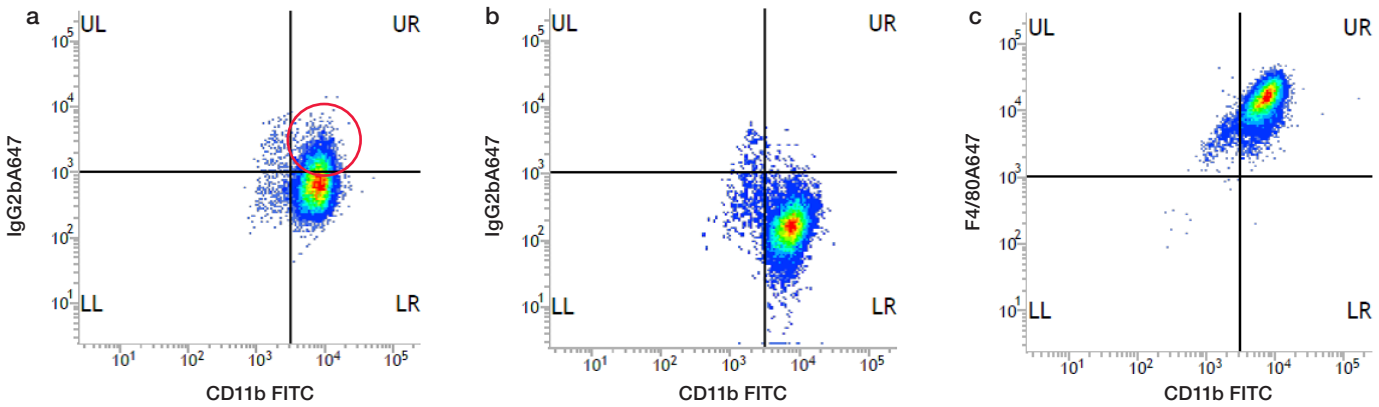


Figure 1. J774 macrophages were stained for 30 minutes at 4°C in PBS w/v1%BSA with CD11b FITC (MCA74F) and IgG2b Alexa Fluor® 647 (MCA6006A647) a). In the absence of Fc block or b). In the presence of mouse Fc block (mouse Seroblock FCR, BUF041A). In Fig. 1a there is a significant population of cells positive (circled) with the A647 isotype control showing the Fc binding. Fig. 1c shows the specific F4/80 A647 (MCA497A647) staining. All data shown was gated on the 7-AAD negative, live population with the use of doublet discrimination.

The role of isotype controls in determining background staining can be observed in Figure 1a where no Fc block has been used (false positive cells circled red) compared to Figure 1b where Fc block has been added. The specific F4/80 staining can be clearly seen in Figure 1c.

The most appropriate isotype control matches:

- The host species
- Ig subclass
- Fluorophore of the primary antibody

As the fluorophore conjugation to the antibody (known as the F/P ratio) can vary between suppliers, it is best to purchase the isotype from the same supplier as the primary. It is also advisable to use them at the same concentration as the primary antibody. Table 1 opposite lists the wide range to be found at Bio-Rad.

Isotype controls have been optimized for surface staining. Intracellular staining can be affected by binding to intracellular components. Intracellular staining may require use of fluorophores other than FITC or RPE, or extra controls to be included, such as unstained, known positive, or known negative samples.

Non-specific antibody binding can be reduced by:

- Blocking Fc receptors
- Adding BSA to your staining buffer
- Titration of your antibody
- Gating out dead cells using a live/dead marker

Remember, isotype controls should not be used to determine compensation levels or the negative population; they are useful to determine how much background you have in your experiment. Single color staining, fluorescence minus one (FMO) and unstained will help with compensation, fluorescence spread and determining gating boundaries when using multiple fluorophores.

Table 1.

	Purified	Biotin	Pacific Blue™	Alexa Fluor 405	Alexa Fluor 488	FITC	RPE	APC	Alexa Fluor 647	RPE-Alexa Fluor 647	RPE-Cy5®	Alexa Fluor 700	PE-Alexa Fluor 750
American Hamster													
IgG	MCA2356	-	-	-	MCA2356A488	MCA2356F	MCA2356PE	-	MCA2356A647	-	-	-	-
Goat													
IgG	STAR153	-	-	-	-	STAR153F	STAR153PE	-	-	-	-	-	-
Mouse													
IgG1	MCA1209	MCA1209B	MCA1209PB	-	MCA1209A488	MCA1209F	MCA1209PE	-	MCA1209A647	-	MCA1209C	MCA1209A700	MCA1209P750
	MCA928	MCA928B	MCA928PB	-	MCA928A488	MCA928F	MCA928PE	MCA928APC	MCA928A647	MCA928A647	-	MCA928A700	MCA928P750
IgG2a	MCA1210	MCA1210B	MCA1210PB	-	MCA1210A488	MCA1210F	MCA1210PE	-	MCA1210A647	-	-	MCA1210A700	-
	MCA929	MCA929B	MCA929PB	-	MCA929A488	MCA929F	MCA929PE	MCA929APC	MCA929A647	MCA929P647	-	MCA929A700	-
IgG2b	MCA691	MCA691B	MCA691PB	-	MCA691A488	MCA691F	MCA691PE	MCA691APC	MCA691A647	MCA691P647	MCA691C	MCA691A700	-
IgG3	MCA5920	-	-	-	-	MCA5920F	MCA5920PE	-	MCA2063A647	-	-	-	-
IgM	MCA692	-	-	-	-	MCA692F	-	-	-	-	-	-	-
Rat													
IgG1	MCA6004	-	MCA6004PB	-	MCA6004A488	MCA6004F	MCA6004PE	-	MCA6004A647	MCA6004P647	-	-	-
	MCA1123R	MCA1123B	-	-	-	MCA1123FT	-	-	-	-	-	MCA1123A700	-
	MCA1211	MCA1211B	-	-	MCA1211A488	MCA1211F	-	-	MCA1211A647	-	-	MCA1211A700	-
IgG2a	MCA6005	MCA6005B	-	-	-	MCA6005F	MCA6005PE	MCA6005APC	MCA6005A647	-	-	-	-
	MCA1124R	-	MCA1124PB	-	MCA1124A488	MCA1124FT	-	-	-	-	-	-	-
	MCA1212	MCA1212B	MCA1212PB	MCA1212A405	MCA1212A488	MCA1212F	MCA1212PE	-	MCA1212A647	MCA1212P647	MCA1212C	MCA1212A700	MCA1212P750
IgG2b	MCA6006	MCA6006B	-	-	MCA6006A488	MCA6006F	-	MCA6006APC	MCA6006A647	-	-	-	-
	MCA1125R	MCA1125B	MCA1125PB	MCA1125A405	-	-	MCA1125PE	-	MCA1125A647	MCA1125P647	-	MCA1125A700	-
IgG2c	MCA2879	-	-	-	-	MCA2879F	-	-	-	-	-	-	-
Syrian Hamster													
IgG	MCA2357	-	-	-	MCA2357A488	MCA2357F	-	-	MCA2357A647	-	-	-	-

For more information visit
bio-rad-antibodies.com/flow-cytometry-isotype-controls

Single Dyes for Flow Cytometry/Microscopy

Fluorophores	Fluorescence Color	Maximal Absorbance (nm)	Maximal Emission (nm)	Relative Brightness
DyLight® 405		400	420	3
Alexa Fluor 405		401	421	3
Pacific Blue		410	455	1
DyLight 488		493	518	4
Alexa Fluor 488		495	519	3
FITC		490	525	3
DyLight 550		562	576	4
PE		496, 546	578	5
Texas Red®		596	615	2
APC		650	661	4
Alexa Fluor 647		650	665	4
Cy5		649	670	3
DyLight 650		654	673	4
PerCP		490	675	2
DyLight 680		692	712	4
Alexa Fluor 700	Infrared	702	723	2
DyLight 755	Infrared	752	778	4
DyLight 800	Infrared	777	794	4

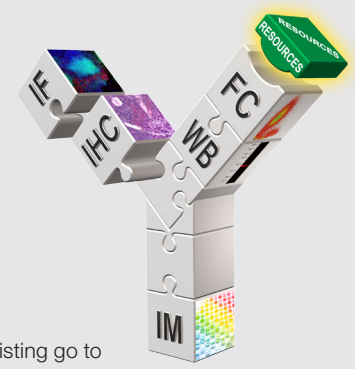
Tandem Dyes for Flow Cytometry

Fluorophores	Fluorescence Color	Maximal Absorbance (nm)	Maximal Emission (nm)	Relative Brightness
PE-Alexa Fluor 647		496, 546	667	4
PE-Cy5		496, 546	667	5
PE-Cy5.5		496, 546	695	4
PE-Alexa Fluor 700	Infrared	496, 546	723	2
PE-Alexa Fluor 750	Infrared	496, 546	779	4
APC-Alexa Fluor 750	Infrared	650	779	4
PE-Cy7	Infrared	496, 546	785	2
APC-Cy7	Infrared	650	785	2

Antibodies for Flow Cytometry

Bio-Rad offers an extensive range of flow cytometry antibodies conjugated to a wide range of fluorophores. Detailed information on flow cytometry products, controls, protocols, fluorophores, tips and tricks are available at **bio-rad-antibodies.com/flow-resources**

- Over 3000 tested antibodies
- 26 fluorophores
- Unique tandem dyes



For full product listing go to
bio-rad-antibodies.com/fc-abs

Visit **bio-rad-antibodies.com/flow-cytometry-isotype-controls** for more information.

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