SPECIALIZING IN

Secondary **Antibodies and Conjugates**

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Jackson ImmunoResearch LABORATORIES, INC.

FAB ANTI-FC FRAGMENT SECONDARY ANTIBODIES

Immunolabel Primary Antibodies in Solution

FABULIGHT™ - SECONDARY ANTIBODIES

Immunolabel Primary Antibodies in Solution

FabuLight antibodies are Fab fragment secondary antibodies specific to the Fc region of IgG or IgM primary antibodies.

They are available conjugated with 9 different fluorophores and biotin, and enable labeling of primary antibodies prior to incubation with cells or tissue. This provides a time-saving alternative to sequential incubation flow cytometry and immunohistochemistry procedures, without compromising the active site of the primary antibody.

FabuLights can be used to label Fc domains of fusion proteins, or cell surface immunoglobulins, without cross-linking or activating cells.

FabuLights are not cross-adsorbed against other species, so blocking steps may be required to avoid labeling endogenous immunoglobulins. For advice on developing protocols, refer to the example protocol overleaf.

Save time and preserve cells by reducing incubation and washing steps.

Incubation with FabuLight-labeled primary antibodies requires fewer washes than sequential incubation with primary antibodies and labeled secondary antibodies, thereby reducing damage to cells in flow cytometry protocols. Incubation steps in protocols requiring multiple primary antibodies from the same host animal are also reduced.

No interference with the primary antibody active site.

FabuLight binds to the Fc portion of the primary antibody (either anti-IgG, Fcγ specific or anti-IgM, μ chain specific), leaving the antigen-binding region active.

No precipitation or aggregation.

The resulting complex of primary antibody with FabuLight does not precipitate or aggregate, because the dye-conjugated Fab anti-Fc secondary antibody fragments are monovalent. FabuLight-primary antibody complexes offer good tissue penetration and low non-specific binding.

	Antibody Description	Unconjugated	Biotin-SP (long spacer)	DyLight™ 405 A=400, E=421	Alexa Fluor® 488	Cyanine Cy™3 A=550, E= 570	Phycoerythrin R-PE A=488, E=580	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667	Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
GOAT	ANTI-CHICKEN											
	Fab Fragment Goat Anti-Chicken IgY (IgG), Fc fragment specific	103-007-008 1.0 mg	103-067-008 0.5 ml	103-477-008 0.5 mg	103-547-008 0.5 mg	103-167-008 0.5 mg	103-117-008 0.5 ml	103-297-008 0.5 mg	103-587-008 0.5 mg	103-607-008 0.5 mg	103-627-008 0.5 mg	103-657-008 0.5 mg
BOVINE	ANTI-GOAT											
	Fab Fragment Bovine Anti-Goat IgG, Fc fragment specific	805-007-008 1.0 mg	805-067-008 0.5 ml	805-477-008 0.5 mg	805-547-008 0.5 mg	805-167-008 0.5 mg	805-117-008 0.5 ml	805-297-008 0.5 mg	805-587-008 0.5 mg	805-607-008 0.5 mg	805-627-008 0.5 mg	805-657-008 0.5 mg
GOAT	ANTI-GUINEA PIG											
	Fab Fragment Goat Anti-Guinea Pig IgG, Fc fragment specific	106-007-008 1.0 mg	106-067-008 0.5 ml	106-477-008 0.5 mg	106-547-008 0.5 mg	106-167-008 0.5 mg	106-117-008 0.5 ml	106-297-008 0.5 mg	106-587-008 0.5 mg	106-607-008 0.5 mg	106-627-008 0.5 mg	106-657-008 0.5 mg
GOAT	ANTI-HUMAN											
	Fab Fragment Goat Anti-Human IgG, Fc_γ fragment specific	109-007-008 1.0 mg	109-067-008 0.5 ml	109-477-008 0.5 mg	109-547-008 0.5 mg	109-167-008 0.5 mg	109-117-008 0.5 ml	109-297-008 0.5 mg	109-587-008 0.5 mg	109-607-008 0.5 mg	109-627-008 0.5 mg	109-657-008 0.5 mg
	Fab Fragment Goat Anti-Human IgM, Fc_μ fragment specific	109-007-043 1.0 mg	109-067-043 0.5 ml	109-477-043 0.5 mg	109-547-043 0.5 mg	109-167-043 0.5 mg	109-117-043 0.5 ml	109-297-043 0.5 mg	109-587-043 0.5 mg	109-607-043 0.5 mg	109-627-043 0.5 mg	109-657-043 0.5 mg
GOAT	ANTI-MOUSE											
	Fab Fragment Goat Anti-Mouse IgG1, Fc_γ fragment specific	115-007-185 1.0 mg	115-067-185 0.5 ml	115-477-185 0.5 mg	115-547-185 0.5 mg	115-167-185 0.5 mg	115-117-185 0.5 ml	115-297-185 0.5 mg	115-587-185 0.5 mg	115-607-185 0.5 mg	115-627-185 0.5 mg	115-657-185 0.5 mg
	Fab Fragment Goat Anti-Mouse IgG2a, Fc_γ fragment specific	115-007-186 1.0 mg	115-067-186 0.5 ml	115-477-186 0.5 mg	115-547-186 0.5 mg	115-167-186 0.5 mg	115-117-186 0.5 ml	115-297-186 0.5 mg	115-587-186 0.5 mg	115-607-186 0.5 mg	115-627-186 0.5 mg	115-657-186 0.5 mg
	Fab Fragment Goat Anti-Mouse IgG2b, Fc_γ fragment specific	115-007-187 1.0 mg	115-067-187 0.5 ml	115-477-187 0.5 mg	115-547-187 0.5 mg	115-167-187 0.5 mg	115-117-187 0.5 ml	115-297-187 0.5 mg	115-587-187 0.5 mg	115-607-187 0.5 mg	115-627-187 0.5 mg	115-657-187 0.5 mg
	Fab Fragment Goat Anti-Mouse IgG2c, Fc_γ fragment specific	115-007-188 1.0 mg	115-067-188 0.5 ml	115-477-188 0.5 mg	115-547-188 0.5 mg	115-167-188 0.5 mg	115-117-188 0.5 ml	115-297-188 0.5 mg	115-587-188 0.5 mg	115-607-188 0.5 mg	115-627-188 0.5 mg	115-657-188 0.5 mg
	Fab Fragment Goat Anti-Mouse IgG3, Fc_γ fragment specific	115-007-189 1.0 mg	115-067-189 0.5 ml	115-477-189 0.5 mg	115-547-189 0.5 mg	115-167-189 0.5 mg	115-117-189 0.5 ml	115-297-189 0.5 mg	115-587-189 0.5 mg	115-607-189 0.5 mg	115-627-189 0.5 mg	115-657-189 0.5 mg
	Fab Fragment Goat Anti-Mouse IgM, μ Chain Specific	115-007-020 1.0 mg	115-067-020 0.5 ml	115-477-020 0.5 mg	115-547-020 0.5 mg	115-167-020 0.5 mg	115-117-020 0.5 ml	115-297-020 0.5 mg	115-587-020 0.5 mg	115-607-020 0.5 mg	115-627-020 0.5 mg	115-657-020 0.5 mg
GOAT	ANTI-RABBIT											
	Fab Fragment Goat Anti-Rabbit IgG, Fc fragment specific	111-007-008 1.0 mg	111-067-008 0.5 ml	111-477-008 0.5 mg	111-547-008 0.5 mg	111-167-008 0.5 mg	111-117-008 0.5 ml	111-297-008 0.5 mg	111-587-008 0.5 mg	111-607-008 0.5 mg	111-627-008 0.5 mg	111-657-008 0.5 mg
GOAT	ANTI-RAT											
	Fab fragment Goat Anti-Rat IgG, Fc_γ fragment specific	112-007-008 1.0 mg	112-067-008 0.5 ml	112-477-008 0.5 mg	112-547-008 0.5 mg	112-167-008 0.5 mg	112-117-008 0.5 ml	112-297-008 0.5 mg	112-587-008 0.5 mg	112-607-008 0.5 mg	112-627-008 0.5 mg	112-657-008 0.5 mg
	Fab fragment Goat Anti-Rat IgM, μ chain specific	112-007-020 1.0 mg	112-067-020 0.5 ml	112-477-020 0.5 mg	112-547-020 0.5 mg	112-167-020 0.5 mg	112-117-020 0.5 ml	112-297-020 0.5 mg	112-587-020 0.5 mg	112-607-020 0.5 mg	112-627-020 0.5 mg	112-657-020 0.5 mg

Technical tips for using FabuLight™.

FabuLight antibodies have not been adsorbed to remove cross-reactivities to other species, so it may be necessary to block endogenous IgG prior to incubation with an unlabeled FabuLight prior to application of a FabuLight-labeled complex.

FabuLight-labeled complexes do not provide as bright a signal as sequential incubation with a primary antibody followed by incubation with a labeled secondary antibody.

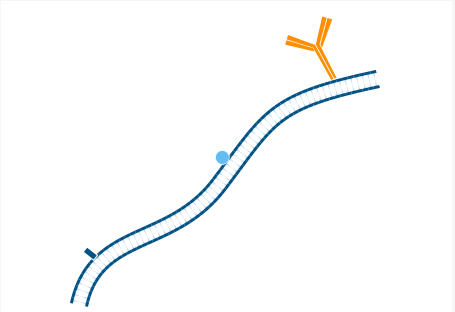
To avoid displacement of the FabuLight-primary antibody by a subsequent labeled secondary antibody, a light cross-linking with glutaraldehyde may be necessary, provided that it does not affect antigenicity of subsequent target proteins.

For all multiple labeling applications, we recommend incubating FabuLights sequentially to minimize cross-reactivity.

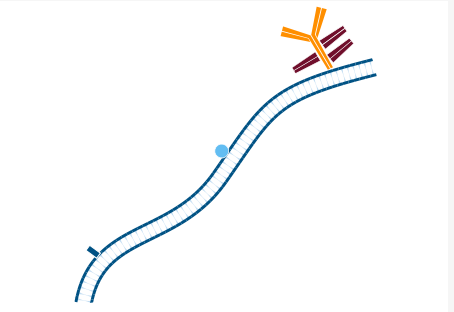
Optimal protocols for each application must be established empirically.

- Label the less abundant target antigen first for best results.
- For IgG class primary antibodies, complexing at a 3:1 molar ratio of FabuLight-primary antibody (equal weight ratios) provides a good degree of labeling of the primary antibody without excessive amounts of unbound FabuLight.
- For IgM primary antibodies, use a 15:1 molar ratio of FabuLight-primary antibody (equal weight ratios).
- Titrate FabuLight-labeled complexes vs. their target antigens to minimize the amount of free Fab anti-Fc, thereby minimizing potential mislabeling in a multiple labeling application.

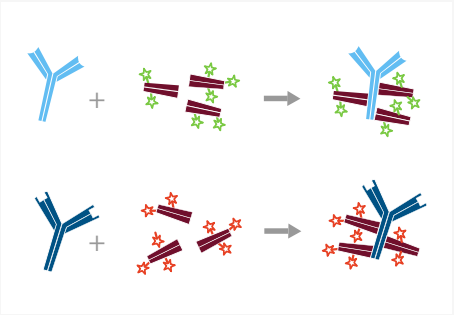
Use of FabuLight - labeled primaries for two antigens on tissue.



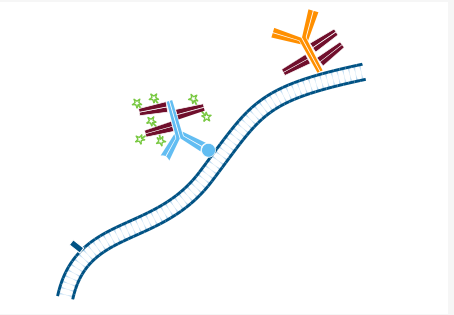
1. Include a blocking step if the tissue of interest displays endogenous immunoglobulin.



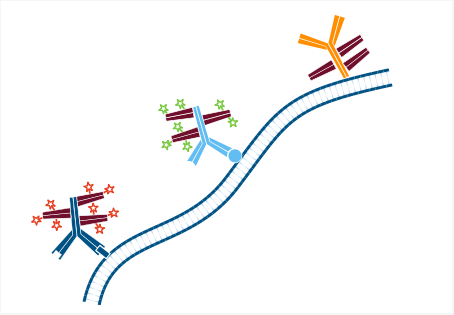
2. In this instance, mouse tissue will be labeled using primary antibodies raised in mouse. Pre-incubate tissue/cells with unconjugated Fab anti-Fc antibody to block endogenous immunoglobulin, in this example Fab Goat Anti-Mouse IgG1, Fcγ fragment specific. Wash.



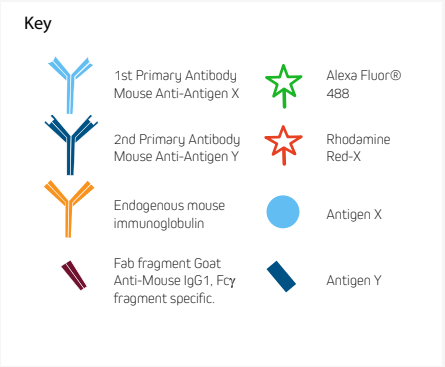
3. Create FabuLight-primary antibody complexes. In this example, Mouse Anti-Antigen X is complexed with Alexa Fluor® 488-conjugated Fab Goat Anti-Mouse IgG1, Fcγ fragment specific (115-547-185); and Mouse Anti-Antigen Y is complexed with Rhodamine Red-X-conjugated Fab Goat Anti-Mouse, IgG1 Fcγ fragment specific (115-297-185).



4. Incubate the sample with Mouse Anti-Antigen X (less abundant target) complexed with Alexa Fluor® 488-conjugated Fab Goat Anti-Mouse IgG1, Fcγ fragment specific. Wash.



5. Incubate the sample with Mouse Anti-Antigen Y (more abundant target) complexed with Rhodamine Red-X conjugated Fab Goat Anti-Mouse IgG1, Fcγ fragment specific. Wash.



The importance of sequential labeling and titrating primary antibodies vs. target antigens.

Figures 1-5 show how optimized conditions can improve multiple labeling protocols. Titrate Fab-labeled complexes vs. their target antigens to minimize interactions from unbound complexes. Figures 1-2 demonstrate that positive signal can be maintained under diluted conditions. Sequential incubations, starting with the less abundant antigenic target, provide the best discrimination between the antigens (cell types) as shown in Figures 3-4. The Fab-labeled complexes can also be incubated simultaneously if they have been properly titrated (Figure 5).

Complexes were formed at a 3:1 molar ratio (equal weight ratios) of FabuLight:primary antibody. Alexa Fluor® 488 Fab Goat Anti-Mouse IgG1, Fcγ fragment specific (115-547-185) was complexed with Mouse Anti-Human CD3 (BD 555330)(**AF488/CD3**); and Alexa Fluor® 647- Fab Goat Anti-Mouse IgG1, Fcγ fragment specific (115-607-185) was complexed with Mouse Anti-Human CD19 (BD 555410)(**AF647/CD19**).

Blood was collected from normal human donors and treated with lysis buffer. Cells were centrifuged, washed, and resuspended in isotonic PBS + 0.5% BSA. They were then incubated with complexed antibodies for 30 minutes, washed, and analyzed on a BD FACSCalibur flow cytometer gated on lymphocytes.

Titrate antibodies to find the lowest dilution that yields the desired population shift.

Figures 1 and 2 illustrate insignificant signal loss with diluted reagents. For multiple labeling protocols, excess reagent will result in mislabeling of antigens as illustrated in Figures 3-5. Use the lowest concentration possible to optimize results.

Figure 1.

Cells were incubated with two different concentrations of **AF488/CD3** and washed. Panels A and B show a large population of T cells in the lower right quadrant, with similar detection at both concentrations.

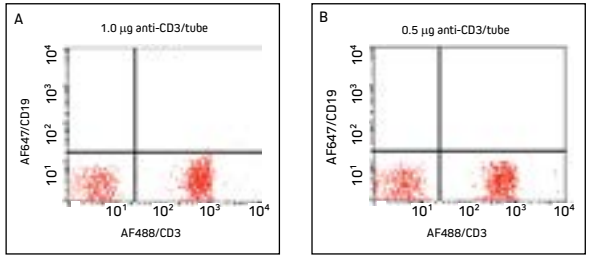
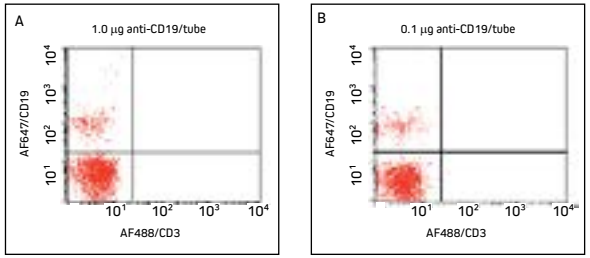


Figure 2.

Cells were incubated with two different concentrations of **AF647/CD19** and washed. Panels A and B show a smaller population of B cells in the upper left quadrant, with similar detection at both concentrations.



Multiple labeling with FabuLights.

Follow the correct steps to ensure the best results with FabuLights.

Figure 3. Cells were incubated with **AF647/CD19** (primary to the less abundant target used first), washed, then incubated with **AF488/CD3** and washed. Note specific labeling of T cells in the lower right quadrant and B cells in the upper left quadrant.

Targeting the less abundant antigenic site first separates the cell populations well. Diluted antibodies (Panel B) produce fewer double labeled cells, with insignificant signal differences.

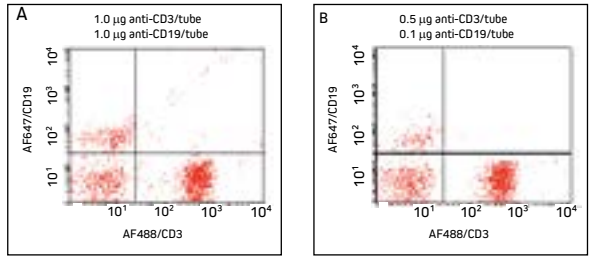


Figure 4. Cells were incubated with **AF488/CD3** (primary to more abundant target used first), washed, then incubated with **AF647/CD19** and washed.

Note increased double labeling of cells compared with Figure 3.

Panel A shows excess AF647-FabuLight has bound to anti-CD3 on the T cells, shifting the population into the upper right quadrant. Panel B shows that under diluted conditions, T cells have shifted down compared with Panel A. Additonal titration of anti-CD19 may further improve this result, though labeling the less abundant target antigen first (Figure 3) avoids the problem.

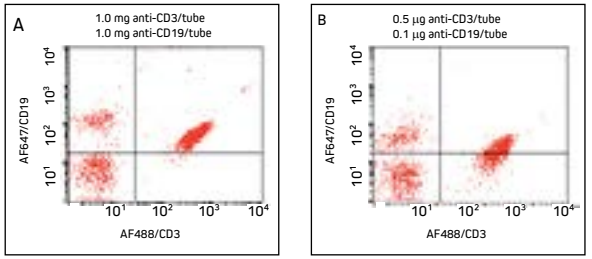


Figure 5. Cells were incubated with a cocktail of **AF488/CD3** and **AF647/CD19** and washed.

Mislabeling of cell populations is dramatically reduced when antibodies are more dilute.

In Panel A, excess AF647-FabuLight has bound to anti-CD3 on T cells, shifting the T cell population into the upper right quadrant. Panel B shows that diluted antibodies bind more specifically, and T cells remain in the lower right quadrant with insignificant loss of signal.