

Fluorochrome Options for Antibody Conjugation

| <u>Single Fluorochrome</u> | <u>Excitation Range</u> | <u>Emission Range</u> | <u>Source Emission</u> | <u>Description</u> |
|---|-------------------------|-----------------------|---------------------------|--|
| FITC (<i>Fluorescein Isothiocyanate</i>) | 468-509nm | 504-541nm | Argon Ion LASER (488nm) | <ul style="list-style-type: none"> ◆ Molecular Weight of 389 daltons. ◆ Fluorescence emission maximum is around 520nm ◆ Using a 530 +/- 15nm band pass filter will give optimal detection. ◆ Usually 2-9 FITC molecules per antibody. |
| R-PE (<i>R-Phycoerythrin</i>) | 486-580nm | 568-590nm | Argon Ion LASER (488nm) | <ul style="list-style-type: none"> ◆ PE is an accessory photosynthetic pigment found in red algae. ◆ 240 kDa protein with 34 phycoerythrobilin fluorochromes per molecule . ◆ Fluorescence emission maximum is around 576nm ◆ Using a 585 +/- 21nm band pass filter (single laser instrument) will give optimal detection. ◆ Usually 1 R-PE molecule per antibody. |
| Percp (<i>Peridinin Chlorophyll Protein</i>) | 490nm max. absorption | 677nm max. emission | Argon Ion LASER (488nm) | <ul style="list-style-type: none"> ◆ PerCP is a component of the photosynthetic apparatus found in the dinoflagellate, Glenodinium sp. ◆ Molecular Weight is 35kDa. ◆ Fluorescence emission maximum is around 677nm ◆ Usually 2-9 PerCP molecules per antibody. ◆ PerCP is highly sensitive to photobleaching. |
| APC (<i>Allophycocyanin</i>) | 600-640nm | 660nm max. emission | Helium-Neon LASER (635nm) | <ul style="list-style-type: none"> ◆ APC is an accessory photosynthetic pigment found in bluegreen algae. ◆ Molecular Weight is about 105kDa with 6 phycocyanobilin chromophores (which are similar in structure to the chromophore in R-PE) per molecule . ◆ Fluorescence emission maximum is around 660nm ◆ Using a 660 +/- 10nm band pass filter will give optimal detection. ◆ Usually 1 APC molecule per antibody. |
| CY 5 | 633nm | 675nm | Helium-Neon LASER (635nm) | <ul style="list-style-type: none"> ◆ CY 5 has less fluorescence intensity than APC. ◆ Cyanines are not water soluble, however are soluble in nonpolar solvents. This characteristic is essential for use as probes of membrane potential, however, is undesirable as a label because it is nonspecifically sticky to protein. |

| <u>Tandem Fluorochrome</u> | <u>Excitation Range</u> | <u>Emission Range</u> | <u>Source Emission</u> | <u>Description</u> |
|----------------------------|-------------------------|-----------------------|------------------------|--------------------|
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| PE-Texas Red <i>(Phycoerythrin-Texas Red-X)</i> – also known as ECD | 486-575nm | 610-635nm | Argon Ion LASER (488nm) | <ul style="list-style-type: none"> ◆ Texas Red is a sulfonyl chloride derivative of sulforhodamine 101. ◆ Molecular Weight is 625kDa. ◆ Excitation of Texas Red is about 595 (same as PE emission) and has a fluorescence emission maximum around 620nm ◆ Using a 620 +/- 10nm band pass filter will give optimal detection. |
| PE-CY5 <i>(Phycoerythrin-Cyanin 5.1)</i> – Also known as PC5, Cy-Chrome and Tricolor | 486-575nm | 660-680nm | Argon Ion LASER (488nm) | <ul style="list-style-type: none"> ◆ PE-CY5 is a tandem conjugate system, combining R-PE (240 kDa) and a cyanine dye (1.5kDa). ◆ Very efficient energy transfer between R-PE and the Cyanine dye (little is lost as 576nm) ◆ Fluorescence emission maximum is around 670nm ◆ Using a 650nm long pass filter will give optimal detection. ◆ Usually 1 PE-CY5 molecule per antibody. |
| PE-CY7 <i>(Phycoerythrin-Cyanin 7)</i> | 486-575nm | 750-810nm | Argon Ion LASER (488nm) | <ul style="list-style-type: none"> ◆ PE-CY7 is a tandem conjugate system, combining R-PE and a cyanine dye. ◆ CY 7 has a fluorescence emission maximum around 767nm. |
| PerCP-CY 5.5 | 490nm max. absorption | 695nm max. emission | Argon Ion LASER (488nm) | <ul style="list-style-type: none"> ◆ PerCP-CY 5.5 is a tandem conjugate system, combining PerCP and a cyanine dye. ◆ Excitation of CY 5.5 is about 675 (same as PerCP emission) and has a fluorescence emission maximum around 695nm |
| APC-CY7 | 600-640nm | 750-810 | Helium-Neon LASER (635nm) | <ul style="list-style-type: none"> ◆ APC-CY7 is a tandem conjugate system, combining APC and a cyanine dye. ◆ CY 7 has a fluorescence emission maximum around 767nm. |

**Alexa
Secondaries
(Molecular
Probes)**

**Excitation
Range**

**Emission
Range**

**Source
Emission**

Description

| | | | | |
|------------------------|-----|-----|---------------------------------|--|
| Alexa Fluor 350 | 347 | 442 | UV LASER (350nm) | <ul style="list-style-type: none"> ◆ Alexa Fluor is a series of dyes from Molecular Probes. Alexa Fluor 350 is UV excitable and emits in the blue region. ◆ Because sample autofluorescence is common in the blue region, Alexa Fluor 350 should be used to label the most abundant targets in multilabeling experiments. |
| Alexa Fluor 488 | 495 | 519 | Argon Ion LASER (488nm) | <ul style="list-style-type: none"> ◆ Alexa Fluor is a series of dyes from Molecular Probes. Alexa Fluor 488 emits in the green region. ◆ Alexa Fluor 488 is significantly brighter than Fluorescein conjugated and are much more photostable. ◆ Alexa Fluor 488 is pH independent between 4-10 (Fluorescein is significantly affected by pH). |
| Alexa Fluor 633 | 632 | 647 | Helium-Neon LASER (635nm) | <ul style="list-style-type: none"> ◆ Alexa Fluor is a series of dyes from Molecular Probes. Alexa Fluor 633 emits in the far-red region which is well beyond the range of most sample autofluorescence.. ◆ Alexa Fluor 633 is bright and photostable. |
| Alexa Fluor 647 | 650 | 668 | Helium-Neon LASER (635nm) | <ul style="list-style-type: none"> ◆ Alexa Fluor is a series of dyes from Molecular Probes. |
| Alexa Fluor 680 | 679 | 702 | Helium-Neon LASER (635nm) | <ul style="list-style-type: none"> ◆ Alexa Fluor is a series of dyes from Molecular Probes. |
| Alexa Fluor 430 | 434 | 540 | 410nm (Krypton) | <ul style="list-style-type: none"> ◆ Alexa Fluor is a series of dyes from Molecular Probes |
| Cascade Yellow | 402 | 545 | 410nm (Krypton) | |
| Cascade Blue | 400 | 420 | 410nm (Krypton) | |