Cytek® Aurora
Fluorochrome Selection Guidelines
3 Laser 16V-14B-8R
Fluorochrome Signatures

Dyes can be used in combination if they have unique spectrum signatures. Look for dyes with unique spectra and consider spread introduced by the dyes when designing multicolor panels (see slide 23).
How to Read Full Spectrum Fluorochrome Signatures

This dye is excited by all 3 lasers. The peak channel (indicated by the black bar) is in channel V11, and it has secondary emission in channels B7 and R1. Based on this information, expect this dye to introduce spread into dyes emitting at similar wavelengths.
Dyes Primarily Excited by the Violet Laser
Violet Laser Excitable Dyes with Similar Signatures (1 of 3)

Super Bright 436 and Zombie Violet

eFluor450, vioBlue, Pacific Blue, and Live/Dead Violet
Violet Laser Excitable Dyes with Similar Signatures (2 of 3)

BV510, VioGreen, Zombie Aqua and Live/Dead Aqua

Pacific Orange and Live/Dead Yellow
Violet Laser Excitable Dyes with Similar Signatures (3 of 3)
Violet Laser Excitable Dyes with Unique Signatures

- Super Bright 436, Zombie Violet
- Live/Dead Blue
- Pacific Blue, eFluor450, VioBlue, Live/Dead Violet
- BV480
- eFluor 506
- BV510, VioGreen, Zombie Aqua, Live/Dead Aqua
- Live/Dead Red
- Pacific Orange, Live/Dead Yellow
- Zombie Red
- Super Bright 600, BV605
- Zombie Yellow
- Qdot605

- Super Bright 645, BV650
- Qdot655
- Super Bright 702
- Qdot705
- BV711
- BV750
- BV785
- Qdot 800

N9-20019 Rev. A
Dyes Primarily Excited by the Blue Laser
Blue Laser Excitable Dyes with Similar Signatures (1 of 2)

BB515, sVio515, and Vio515

Alexa Fluor 488, FITC, vioBright FITC, Zombie Green
Blue Laser Excitable Dyes with Similar Signatures (2 of 2)

PE/Dazzle594, PE-eFluor 610 and PE-Texas Red

PerCP vio700 and PerCP-eFluor 710

PE Vio770 and PE-Cy7
Blue Laser Excitable Dyes with Unique Signatures

- BB515, sVio515, Vio515
- Alexa Fluor 488, FITC, vioBright FITC, Zombie Green
- Live/Dead Green
- Alexa Fluor 532
- PE/Cy5
- PE-Cy5.5
- PerCP
- PE
- PE/Cy7
- PE/Dazzle 594, PE-CF594, PE-eFluor 610, PE-Texas Red
- PE/Vio770, PE-Cy7
- PerCP vio700, PerCP-eFluor 710
- PerCP-Cy55

N9-20019 Rev. A
Dyes Primarily Excited by the Red Laser
Red Laser Excitable Dyes with Similar Signatures

eFluor 660, Alexa Fluor 647, Vio 667, sVio 667 and Live/Dead Far Red
Red Laser Excitable Dyes with Similar Signatures

APC-Alexa 750, APC-Vio 770, APC/Fire 750, APC-Cy7, APC-eFluor 780, APC-H7, and Live/Dead NIR
Red Laser Excitable Dyes with Unique Signatures

- APC-eFluor 660, Alexa Fluor 647, Vio667, sVio 667, Live/Dead Far Red
- APC-Cy5.5
- Alexa Fluor 700
- APC-R700
- APC-Alexa Fluor 750, APC/Fire 750, APC-Vio 770, APC-eFluor 780, APC-H7, Live/Dead NIR
- Zombie NIR
Peak Channels & Possible Combination of Dyes
# Fluorochrome Peak Channels

## Violet Excited Fluors

<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>Peak Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV421, Alexa Fluor 405, Super Bright 436, Zombie Violet, Live/Dead Blue</td>
<td>V1</td>
</tr>
<tr>
<td>eFluor 450, VioBlue, Pacific Blue, Live/Dead Violet</td>
<td>V2</td>
</tr>
<tr>
<td>eFluor 506</td>
<td>V5</td>
</tr>
<tr>
<td>BV510, VioGreen, Zombie Aqua, Live/Dead Aqua</td>
<td>V7</td>
</tr>
<tr>
<td>BV570, Pacific Orange, Live/Dead Yellow</td>
<td>V8</td>
</tr>
<tr>
<td>BV605, Super Bright 600, Qdot 605, Live/Dead Red, Zombie Yellow</td>
<td>V10</td>
</tr>
<tr>
<td>BV711, Super Bright 702, Qdot 705</td>
<td>V11</td>
</tr>
<tr>
<td>BV750</td>
<td>V13</td>
</tr>
<tr>
<td>BV785, BV786, Qdot 800</td>
<td>V15</td>
</tr>
</tbody>
</table>

## Blue Excited Fluors

<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>Peak Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB515, sVio515, Vio515</td>
<td>B1</td>
</tr>
<tr>
<td>Blue Excited Fluor 488, FITC, VioBright FITC, Zombie Green</td>
<td>B2</td>
</tr>
<tr>
<td>Blue Excited Fluor 532, Live/Dead Green</td>
<td>B3</td>
</tr>
<tr>
<td>PE, PE/Cy5, PE-Cy5.5, PerCP</td>
<td>B4</td>
</tr>
<tr>
<td>PE-Dazzle 594, PE-CF594, PE-eFluor 610, PE-Texas Red</td>
<td>B6</td>
</tr>
<tr>
<td>PE-Cy5.5, PerCP-Cy5.5, BB700</td>
<td>B9</td>
</tr>
<tr>
<td>PerCP Vio700, PerCP-eFluor 710</td>
<td>B10</td>
</tr>
<tr>
<td>PE Vio770, PE-Cy7</td>
<td>B13</td>
</tr>
</tbody>
</table>

## Red Excited Fluors

<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>Peak Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>R1</td>
</tr>
<tr>
<td>APC-Cy5.5</td>
<td>R3</td>
</tr>
<tr>
<td>APC-Alexa Fluor 700, APC-R700</td>
<td>R4</td>
</tr>
<tr>
<td>APC-Alexa Fluor 750, APC/Fire 750, APC-Cy7, APC-Vio 770, APC-eFluor 780, APC-H7, Live/Dead NIR</td>
<td>R7</td>
</tr>
</tbody>
</table>
Example of 24 Dyes that Can Be Used in Combination (CAREFUL PANEL DESIGN IS NEEDED)

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Fluorophore</th>
<th>Fluorophore</th>
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<tbody>
<tr>
<td>BB515</td>
<td>APC</td>
<td>BV421</td>
</tr>
<tr>
<td>Alexa Fluor 488 or FITC</td>
<td>Alexa Fluor 647</td>
<td>Super Bright 436</td>
</tr>
<tr>
<td>Alexa Fluor 532</td>
<td>APC-R700 or AF700</td>
<td>eFluor 450 or equivalent</td>
</tr>
<tr>
<td>PE</td>
<td>APC/Fire 750 or equivalent</td>
<td>BV480</td>
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<tr>
<td>PE/Cy5</td>
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<tr>
<td>PerCP-Cy5.5</td>
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<tr>
<td>PerCP-eFluor710</td>
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<tr>
<td>PE-Cy7</td>
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</tbody>
</table>
Stain Indexes

Data generated using CD4 staining in human PBMCs
Stain Index Ranking - 59 Dyes

Pacific Orange
Alexa Fluor 532
Qdot 705
Qdot 605
PerCP-Alexa Fluor 610
eFluor 568
Alexa Fluor 405
BV510
Qdot 655
BV650
PerCP-Cy5.5
PerCP-Vio700
PE-Texas Red
APC-Alexa Fluor 750
Alexa Fluor 700
Pacific Blue
eFluor 450
PE-Alexa Fluor 700
FITC
VioGreen
Super Bright 645
APC-Ch7
BV605
BV650
APC-Fluor 750
Super Bright 660
Alexa Fluor 488
APC-Ch7
Super Bright 702
VioBlue
APC-Fluor710
VioBight
PE/Dazzle 594
BV480
BV750
BV711
BV515
BV235
APC-Fluor 780
PE-Fluor 610
APC-Vio770
Super Bright 486
Vio677
APC
PE-Cy5
PE-Cy7
eFluor 660
PE-Fluor770
BV421
BV667
PE-Cy5.5
APC-R700
B700
Alexa Fluor 647
Vio515
Stain Index Violet Excitable Dyes

Stain Index Blue Excitable Dyes

Stain Index Red Excitable Dyes
Cross-Stain Index Matrix

Dyes used in combination need to have unique spectra AND need to be assessed in terms of spread that they introduce to other dyes.

For example PerCP-Cy5.5 and PE-Cy5.5 have distinct signatures, but since both dyes emit in the same wavelength range and significant spread is introduced by PE-Cy5.5, careful panel design is needed when used in combination.
Spread Matrix for 24 Fluors that can be Used in Combination

<table>
<thead>
<tr>
<th>BV421</th>
<th>Super Bright 436</th>
<th>eFlour 450</th>
<th>BV480</th>
<th>BV510</th>
<th>BV570</th>
<th>BV605</th>
<th>BV711</th>
<th>BV750</th>
<th>BV785</th>
<th>BB515</th>
<th>Alexa Fluor 488</th>
<th>Alexa Fluor 532</th>
<th>PerCP-Cy5.5</th>
<th>PerCP-eFluor 710</th>
<th>PE</th>
<th>PE-Dazzle594</th>
<th>PE-Cy5</th>
<th>PE-Cy7</th>
<th>APC</th>
<th>Alexa Fluor 647</th>
<th>Alexa Fluor 700</th>
<th>APC-Fire 750</th>
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To read this table: fluor in the row impacts the one in the column. Red means the fluor in that row has significant spread into the dye in the column (for example PE into BV570). Areas in bright pink and red is where more attention to panel design is needed.
## Document Revision History

<table>
<thead>
<tr>
<th>Effective Date</th>
<th>Description of Change</th>
<th>Revision</th>
<th>EC No.</th>
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<tbody>
<tr>
<td>10/21/2019</td>
<td>Initial Release</td>
<td>A</td>
<td>EC-00265</td>
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