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**CD127-BV650 (clone HIL-7R-M21 ) from BD (cat#563225) often gives poor signal**

→ Difficult to gate out CD25+CD127-/low cells

**Purpose:** Compare to alternative from Biolegend, CD127-BV650 clone A019D5, cat# 351326)

<table>
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<tr>
<th>Standard panel</th>
<th>Adjusted panel</th>
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<tbody>
<tr>
<td>1. CD3 V500</td>
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<td>2. CD8 PerCPCy5.5</td>
<td>2. CD8 PerCPCy5.5</td>
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<tr>
<td>3. CD4 AF700</td>
<td>3. CD4 AF700</td>
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<td>4. CD25 PECY7</td>
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<td><strong>5. CD127 BV650 BD</strong></td>
<td><strong>5. CD127 BV650 Biolegend</strong></td>
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<tr>
<td>6. Foxp3 PECF594</td>
<td>6. Foxp3 PECF594</td>
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<tr>
<td>7. CD45RA-APC H7</td>
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Gating strategy

Reference plots for CD25/CD127 gating

Old CD127 antibody (BD, cat# 563225; clone HIL-7R-M21)

New CD127 antibody (Biolegend, cat# 351326; clone A019D5)
With old CD127 antibody (BD, cat# 563225; clone HIL-7R-M21)

Foxp3 = 17.3 % of CD4
CD25+CD127-Foxp3+ cell = 2.6% of CD4

With new CD127 antibody (Biolegend, cat# 351326; clone A019D5)

Foxp3 = 59.3 % of CD4
CD25+CD127-Foxp3+ cell = 2.5% of CD4
conclusions:

• CD127 BV650 antibody from Biolegend much better than from BD
• Adjusted gating:
  → % CD25posCD127low/neg population within CD4 greatly reduced
  → FoxP3pos population within CD25posCD127low/neg population greatly increased

→ Frequency of Foxp3+ cells within CD4 similar!!