The chemokine receptor CXCR4 has multiple critical functions in normal physiological processes involving embryonic development of the cardiovascular, hematopoietic and central nervous systems. It has also been implicated in disease pathologies such as HIV infection, cancer metastasis, leukemia progression and rheumatoid arthritis, and is fueling the search for small molecule CXCR4 antagonists as a means for intervention. Here we demonstrate the suitability of the Tag-lite HTRF technology for CXCR4 ligand binding assays for high throughput screening applications. Automated assay performance and pharmacology will be presented.

**Materials and Methods**

**Assay Materials**
- CXCR4 receptor binding kit materials including:
  - Tag-lite CXCR4 receptor red agonist (L0012RED)
  - Tag-lite CXCR4 labeled cells (C11T11CXCR4)
  - Tag-lite buffer (LABMED)
- AMD 3100, antagonist (used in the competition assay, and as the non-specific binding signal referred to as REF1 in the assay protocol)
- SDF1a, unlabelled, agonist (used as compound X in the competition assay for Z' validation)
- Greiner BioOne plates Cat.No. 784075 (384-well, small volume, PolySystene, medium binding, white)

**Automation**
- Synergy H1 Hybrid Multi-Mode Microplate Reader equipped with Terbium (Tb) detection based HTRF module
- Emission filters: 620/10, 665/8
- Excitation filter: 340/30
- Dichroic Mirror: 400nm 1/2

**MultiFlo Microplate Dispenser technology**
- MultiFlo Microplate Dispenser technology equipped with a 1 µL peristaltic pump cassette provided automated dispensing of cells and fluorescent labeled ligand

**Results**

**Table 1 – Automated Z' data**

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD3100</td>
<td>30.0</td>
<td>29.5</td>
<td>29.2</td>
<td>29.1</td>
</tr>
</tbody>
</table>

**Table 2 – Pharmacology Data Comparison**

1. MultiFlo Microplate Dispenser technology is suitable for HTS applications with the Tag-lite HTRF assay method.
2. Each MultiFlo unit can be equipped with up to 2 peristaltic and 2 syringes pumps for a wide range of liquid dispensing from the same small footprint.
3. Precision XS Microplate Pipetting System is adept in automating the serial dilutions necessary for conducting dose response curves to evaluate ligand binding pharmacology evaluations.
4. Synergy H1 Hybrid Multi-Mode Microplate Reader provides high performance, cost-effective HTRF detection.