**Introduction**

The development of monoclonal antibodies relies on biochemical or cell-based screens to identify binders with high affinity and specificity to the target. For simplicity and throughput, cell culture supernatants in primary screens are usually tested at a fixed volume or dilution, without prior normalisation of the sample concentration. However, it is important to note that the binding signal measured in such assays depends on both the affinity and the concentration of the sample, which can lead to ambiguous data if there is a large discrepancy between the two (Table 1).

Table 1 The effect of antibody affinity and concentration on the screening assay signal

<table>
<thead>
<tr>
<th>Affinity</th>
<th>Concentration</th>
<th>Assay signal</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Strong hit</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>Medium</td>
<td>Medium hit (risk false negative)</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
<td>Medium</td>
<td>Medium hit (risk false positive)</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>No hit</td>
</tr>
</tbody>
</table>

A screening method that enables the determination of antibody concentration (titre) at the same time as binding affinity would therefore be highly desirable for better hit picking in primary screens. Such assays would also be beneficial at later stages of the discovery pipeline, for example to verify the expression of binders following reformatting into mammalian expression vectors or to select the best antibody secreting cell lines after expression optimisation.

Here we describe the development of a simple bead-based assay to determine the antibody concentration of human, mouse or rabbit samples on the mirrorball fluorescence cytometer. The assay format is completely homogenous for ease of use and may be multiplexed with cell or bead-based binding assays (see app note: mirrorball: simultaneous determination of antibody binding, specificity and titer on the mirrorball fluorescence cytometer.)

**Key Benefits**
- Full length IgG determination
- Option to multiplex with bead and cell based assays
- No-wash protocol
- Compatible with high density microplates & simple liquid handling stations

Fig 1: assay principle:
Recombinant IgG standard or cell culture supernatant containing soluble IgGs is mixed with anti-IgG capture beads and detection antibody on 384-well microplates. During the incubation period, the IgG sample binds to the capture beads and is in turn bound by the fluorescent detection reagent. mirrorball identifies the anti-IgG capture beads based on their intrinsic red fluorescence and then measures the amount of green detection antibody associated with that bead, which is proportional to the sample concentration. A standard curve is used to calibrate the assay and utilised to determine the concentration of unknown IgG samples in cell culture supernatants.
### Materials
- sol-R 5 streptavidin-coated beads (TTP Labtech, #4150-09041)
- IgG standards (table 1)
- Capture and detection antibody (table 1)

### Method
1. Coat sol-R beads with 2-10 μg/mL of the biotinylated capture antibody
2. Prepare detecting mix according to the table below (quantities sufficient for 1 x 384-well microplate)

<table>
<thead>
<tr>
<th>Component</th>
<th>detection mixture (2x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-IgG coated sol-R capture beads</td>
<td>2 x 10⁶ beads</td>
</tr>
<tr>
<td>Detection antibody</td>
<td>800 ng/mL</td>
</tr>
<tr>
<td>Cell culture media</td>
<td>to 4 mL</td>
</tr>
</tbody>
</table>

3. To each well of the assay plate add 10 μL of recombinant IgG standard, then overlay with a further 10 μL of detecting mixture
4. Cover the microplate with a seal or lid and incubate for at least 2 hours at room temperature
5. Read plate on the mirrorball fluorescence cytometer

### Results
**Standard curves**

Fig 3. human, mouse and rabbit IgG titer standard curves used for assay calibration

### Summary
Here we have presented a simple homogenous binding assay for antibody concentration/titre determination. The homogenous (no-wash) assay format minimises the “hands-on” time for the operator and is compatible with simple liquid handling dispensers for setup. These features make this method ideally placed to accelerate the pace of antibody screening, whilst minimising the costs associated with setting of multiple washed assays.

### About Mirrorball
The mirrorball plate-based fluorescence cytometer uses TTP Labtech’s laser scanning technology to provide HTS-friendly workflows that deliver gold standard data quality. The mirrorball’s proprietary background rejection optics enable the use of streamlined no-wash assay formats for multiplexed cell-, and bead-based applications that provide process efficiencies over standard ELISA and flow cytometry.