**Comparison of calcium flux assays across multiple GPCRs: implications for compound profiling and screening**

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**Introduction**
Assays of GPCR activation are widely used employing fluorescence on Fluo-4® and Fluo-3® dyes. Several commercial kits facilitate re-use protocols that are considered to be more robust than those requiring wash steps. Presently, no-wash (NW) kit techniques are often employed, while the use of Fluo-4® and Fluo-3® has diminished. NW kits often use a masking dye to reduce background fluorescence. Two main approaches are employed by no-wash kits. One eliminates washing by addition of a masking dye to reduce background fluorescence. To date, limited data has been presented comparing these two approaches against a large panel of GPCR targets.

**Multispan technology and protocol**
Calcium flux assays require a transcription factor and receptor expression. The expression of the GPCR receptor is achieved by co-transfection with a Fluo-4® or Fluo-3® reporter dye. Raw traces from the Multispan® Calcium Assays can be used to assess the efficacy of the Fluo-4® NW kit, which is often used to identify hits in high-throughput screening approaches. The raw traces from the Multispan® Calcium Assays can be used to assess the efficacy of the Fluo-4® NW kit, which is often used to identify hits in high-throughput screening approaches.

**Raw traces GPCR 1-16**

**Raw traces GPCR 17-32**

**Raw traces GPCR 33-48**

**Calcium flux kit comparison in stable cell lines**

**Table 1: EC₅₀ and IC₅₀ values for GPCRs**

**Summary**
- Multispan® technology enables the development of high-throughput calcium flux assays using Fluo-4® and Fluo-3® dyes. The use of Fluo-4® and Fluo-3® has diminished. NW kits often use a masking dye to reduce background fluorescence. Two main approaches are employed by no-wash kits. One eliminates washing by addition of a masking dye to reduce background fluorescence. To date, limited data has been presented comparing these two approaches against a large panel of GPCR targets.

**Figure 1: The calcium assay kits from Molecular Devices employ neutralised calcium and calcium in A and together with masking dye.**

**Figure 2: The raw traces from the Multispan® Calcium Assays can be used to assess the efficacy of the Fluo-4® NW kit, which is often used to identify hits in high-throughput screening approaches.**

**Table 2: EC₅₀ and IC₅₀ values for GPCRs**

**Figure 3: The raw traces from the Multispan® Calcium Assays can be used to assess the efficacy of the Fluo-4® NW kit, which is often used to identify hits in high-throughput screening approaches.**

**Figure 4: The raw traces from the Multispan® Calcium Assays can be used to assess the efficacy of the Fluo-4® NW kit, which is often used to identify hits in high-throughput screening approaches.**

**Figure 5: The raw traces from the Multispan® Calcium Assays can be used to assess the efficacy of the Fluo-4® NW kit, which is often used to identify hits in high-throughput screening approaches.**