**Miniaturized Kinase uHTS In 1536 Well Format Using IMAP Technology In An Imager.**

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**Introduction**

Kinases have become a proven target class for new drugs and have created a thirst for the development of new technology platforms for inhibitor detection. Some of these provide a generic approach, whereas others are specific to discrete kinase families. New approaches, such as IMAP technology, to the screening of clinical programs are therefore essential to ensure the robustness and reproducibility of the process.

**Materials & Methods**

Reactions were performed in 96-well black flatbottom plates at 37°C in 25mM Hepes, 25mM NaCl, 2mM MgCl2, 2mM ATP, 0.01% Tween-20, 0.15mg/ml BSA and 200uM fluorescent substrate. The IMAP Progressive Binding Reagent was comprised by 10% buffer A and IMAP buffer B 7:3. The assay buffer in Kinase 1 was 25mM Hepes, 25mM NaCl, 2mM MgCl2, 2mM ATP, HEPES, DTT, 0.01% Tween-20, BSA Fraction V and NaCl were from Molecular Devices.

**Assay Principle**

IMAP technology is a novel method for the screening of kinase inhibitors. The fluorescent substrate binds to the large M(III)-based nanoparticles which reduces the rotational speed of the substrate and thus increases its polarization. The Assay Buffer in Kinase 1 was 25mM Hepes, 25mM NaCl, 2mM MgCl2, 2mM ATP, HEPES, DTT, 0.01% Tween-20, BSA Fraction V and NaCl were from Molecular Devices.

**HITS Campaigns**

The full dose screening campaigns were run in parallel, using the same plate configuration. Each Kinase 1 or Kinase 2 project involved 500 different compounds at a single point in a total of 10 working days. Each campaign was comprised by 10% buffer A and IMAP buffer B 7:3. The Kinase buffer in Kinase 1 was 25mM Hepes, 25mM NaCl, 2mM MgCl2, 2mM ATP, HEPES, DTT, 0.01% Tween-20, BSA Fraction V and NaCl were from Molecular Devices.

**Quality Assessment & Process Evolution of the HITS campaigns**

The sensitivity of the miniaturized assay conditions was checked by measuring standard compounds' plate-to-plate.

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**Conclusion**

Kinase 1 and Kinase 2 were carried out in a 10K compound campaign, where approximately 10K compounds were tested at 10uM in three separate experiments, and results showed good performance and reproducibility interdays with correlation coefficients close to 0.9. These results were very similar with the Kinases 1 & 2, therefore we only show the data analysis of a representative one. The sensitivity of the miniaturised assay conditions was checked by measuring standard compounds' plate-to-plate.

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