Development, Automation and Miniaturization of High-Throughput Serine/Threonine Kinase Assays Using the LANCE® Ultra Platform
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1 Introduction
Protein kinases are involved in the regulation of many aspects of the cell cycle, including proliferation, differentiation, secretion and apoptosis. Altered protein kinase expression or functioning is a cause or consequence of many human diseases. As a result, kinases are attractive therapeutic targets for drug discovery programs and this has prompted the development of many kinase assay technologies suitable for high-throughput screening (HTS). In the new LANCE Ultra platform, the classical APC acceptor dye has been replaced by the new small molecular weight red-shifted dye UltraLight™ which allows direct labeling of peptide substrates. This enables the use of fewer assay components, simplifying assay set-up, whilst maintaining very low compound interference rates typically seen using europium chelate and red-shifted emission dye TR-FRET pairs. In this poster, we initially present data describing the development and optimization of two LANCE Ultra serine/threonine kinase (Ser/Thr) assays in a 384-well format using manual pipettors. We then show the automation and miniaturization of both kinase assays to demonstrate the potential of the LANCE Ultra technology, combined with the JANUS® Modular Dispense Technology™ (MDT) Automated Workstation, to reduce assay costs while maintaining HTS robustness.

2 Assay Principle
In LANCE Ultra kinase assays, the phosphorylation of a UltraLight peptide substrate is detected with a specific anti-phosphopeptide antibody (Ab) labeled with europium chelate chelate molecules (Eu). The binding of the Eu-antibody (donor) to the phosphorylated UltraLight peptide substrate brings both the donor and acceptor dye molecules into close proximity. Upon irradiation at 320 nm, the excited europium chelate transfers its energy to the nearby UltraLight dye molecule that will in turn emit light at 665 nm. The intensity of light emission is proportional to the level of the UltraLight peptide phosphorylation.

3 General Protocol for Manual Assay

4 IKKβ Manual Assay Development

5 Akt1/PKBα Manual Assay Development

6 Assay Conditions for Automated Assays

7 Intra-Plate Variability in Low-Volume 384-well Format (1h Detection Time)

8 Intra-Plate Variability in Low-Volume 384-Well Format (O/N Detection Time)

9 Intra-Plate Variability in 1536-Well Format (1h Detection Time)

10 Inter-Plate Variability Assessment

11 Automated Workstation

12 Summary and Conclusions

- Two Ser/Thr LANCE Ultra kinase assays were initially developed and optimized in 384-well plates (20 µL assay) using manual pipettors. Both assays were found to be suitable for HTS purposes as illustrated by Z'-factors of 0.80 (IKKβ) and 0.82 (Akt1).
- Automation and miniaturization to the low-volume 384-well (10 µL assay) and 1536-well (5 µL assay) formats were then conducted by maintaining final concentration of reagents in both the kinase reaction and detection steps.
- Automated and miniaturized assays showed a satisfactory assay quality for both the low-volume 384 and the 1536-well formats (Z' above 0.7 and 0.6, respectively).
- The current preliminary data demonstrate that the LANCE Ultra platform, combined with the JANUS MDT Automated Workstation, is capable of automated and substantial miniaturization of Ser/Thr kinase assays with the potential for reduction of reagents without compromising assay quality.
- Further work will include testing of the JANUS MDT Nanolab™ dispenser head combined with new 1536-well plates in order to reduce assay volume to 2.5 µL.

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