Take advantages of HTRF to Simplify HTS Cascade

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Abstract

HTRF assay is homogeneous, very sensitive, and easy to miniaturize. So, it has been used in HTS frequently. Its features of time-resolved and longer emission wavelength, as well as ratio-metric readout result in little potential interference from colored compounds.

Taking these advantages of this assay format, we developed a HTRF assay with high performance for a given target and then simplified the HTS cascade by eliminating single-poke confirmation and the artifact screening.

With this simplified cascade, we delivered final HTS results with excellent outcome in less time and fewer cost.

Normal HTS *vs* Simplified HTRF HTS

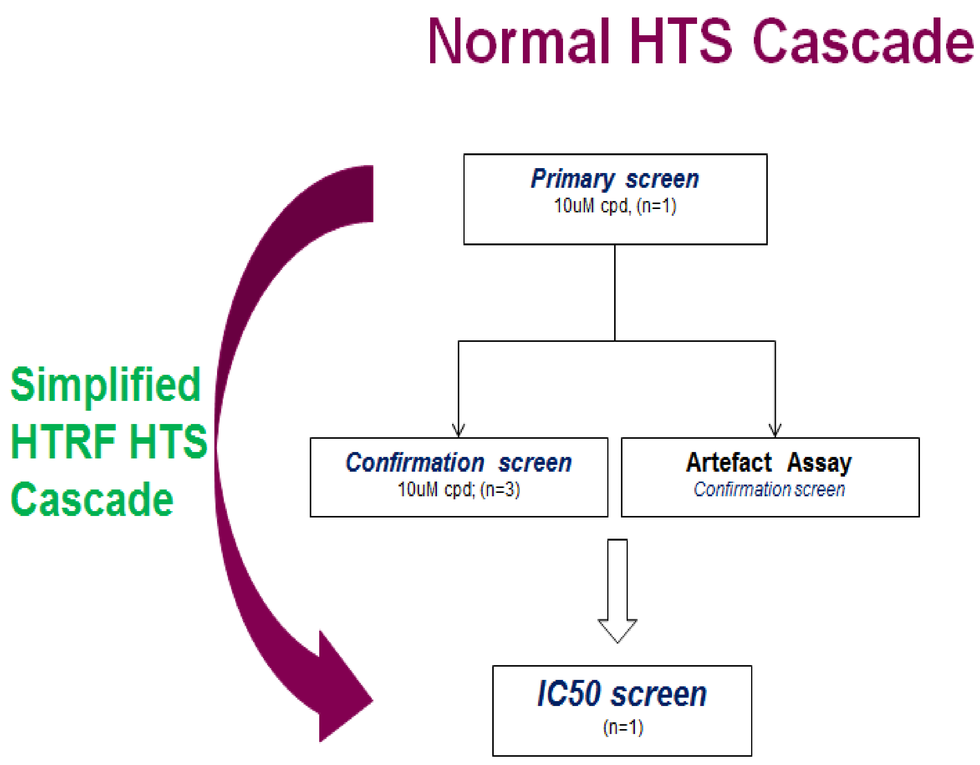

Figure1. Comparison between normal and HTRF HTS cascade

Introduction

➢*Assay Description*

HTRF KinEASE™ – TK is a generic method for measuring tyrosine kinase activities using one substrate and a universal detection system.

✓**Enzymatic step**: During this step, the kinase will phosphorylate the substrate. The TK Substrate – biotin is incubated with the kinase. ATP is added to start the enzymatic reaction.

✓**Detection step**: The detection reagents will catch the phosphorylated substrate. The resulting TR-FRET signal is proportional to the phosphorylation level. The TK-Antibody labeled with Eu3+ - Cryptate and streptavidin – XL665 are then added with EDTA (used to stop the kinase activity).

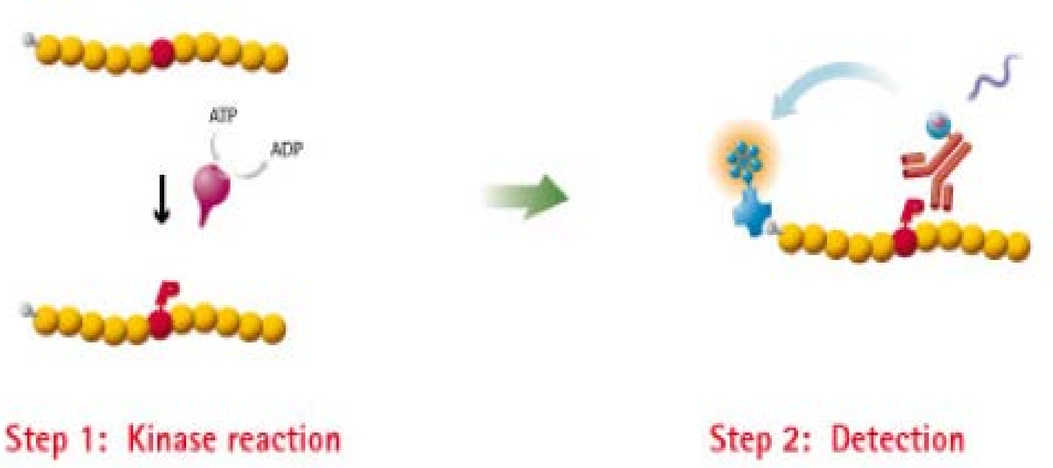

Figure 2. Principle of the HTRF® KinEASE™ - TK assay.

Platform Validation and HTS

HTS Assay Validation

- ✓ Well2well: ~6% CV
- ✓ Plate2plate & Day2day variations: Reference IC50 < 2x
- ✓ Reference IC50 and S/N (~5): Stable within 24h
- ✓ The 3SD cut-off: ~25%.
- ✓ Double blind test: Captured 40 points of > 30% inhibition with 100% match with the compound map (10 pts of each IC30, 40, 50, 60 in random positions: total 40 points)

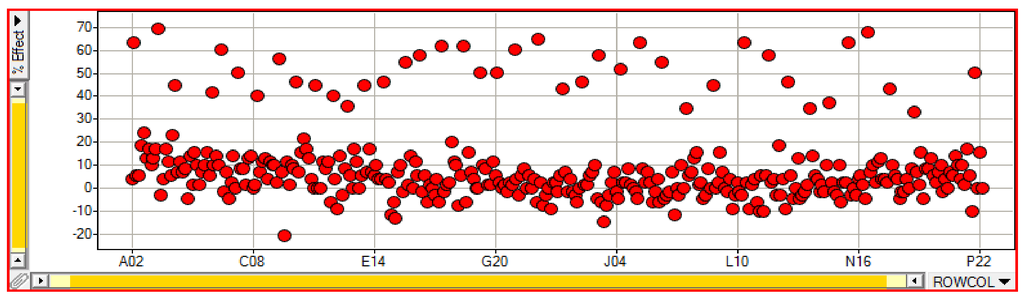

Figure3. Results of double blind test

Robust HTS Performance

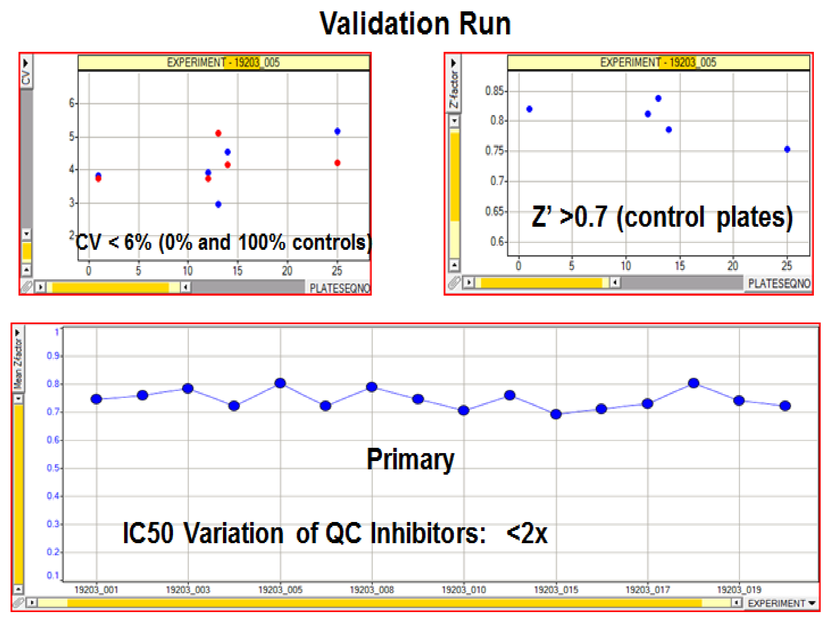

Figure4. Quality Control of HTS performance

Results of HTS

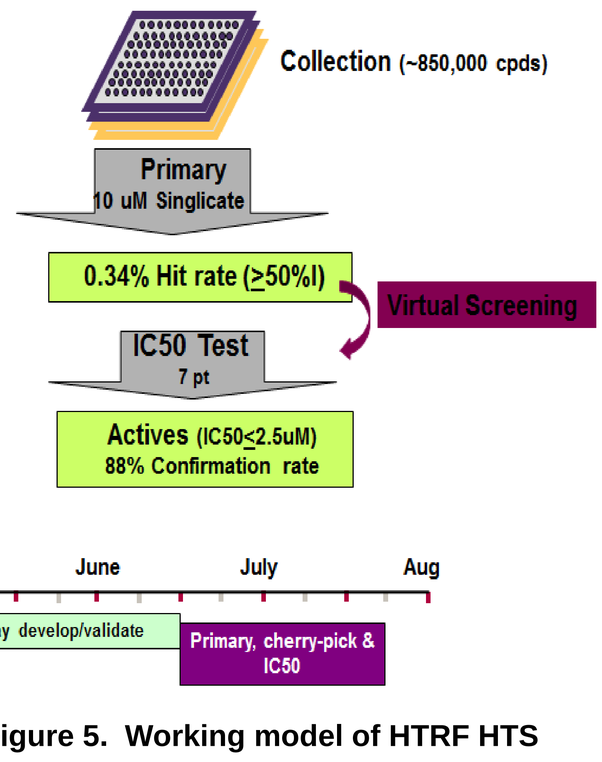

Figure 5. Working model of HTRF HTS

Conclusions

- ➢ **HTRF has very low artifact rate:** Artifact screening won't add significant value.
- ➢ **HTRF has excellent performance and thus high confirmation rate:** Confirmation screening may not need.
- ➢ **Therefore, simple cascade may be applied to HTS using HTRF:** Save time and money.