Kinase assay unaffected by luciferase inhibition in HTS in compound libraries
Jonas Eriksson, Robert Inglis, Arne Lundin*; BioThema AB

Introduction
Assays of protein kinase activity using the firefly luciferase reaction are available from several companies. In these assays ATP remaining after a predefined incubation period is measured. Such assays are, however, affected by inhibition of luciferase by as much as 3% of library compounds. This can result in false negatives in HTS. We have solved the problem by performing the kinase reaction in the presence of luciferase and luciferin as described under “Assay principles”. In this way the kinase activity cancels out and inhibition is no longer a problem.

Effect of ATP Concentration

![Graph showing the effect of ATP concentration on light emission.](Fig. 1: Normalised light emission at (from top to bottom) 32, 16, 8, 4, 2, 1 and 0.5 µM ATP PKA (0.125 ng/µL) with Kemptide (23 µM) as substrate.)

**Z’ as High as 0.96**

![Graph showing Z’ values and remaining ATP as a function of time.](Fig. 3: Z’ value and remaining ATP as a function of time. 40 blanks (no PKA) and 40 samples (with PKA) were measured. An average blank curve was used to calculate individual normalised blank and sample curves from which Z’ values were calculated. A maximum Z’ (0.96) is obtained already when ATP has gone down one order of magnitude.)

Assay Principles

- **Real-time measurement of ATP consumption by having luciferase and luciferin present during kinase reaction.**
  \[ \text{ATP} + \text{peptide} \rightarrow \text{ADP} + \text{PPi} + \text{luxinyl} + \text{CO}_2 + \text{light} \]
- Negligible ATP consumption in luciferase reaction
- Variations in ATP and inhibitor level for HTS
- Kinase activity cancels out in the calculations
- The first order rate constant, \(k\), is used as a measure of kinase activity, where \(I_1\) and \(I_2\) are normalised light emission at times \(t_1\) and \(t_2\)
- Standard Curve for PKA

![Graph showing the standard curve for PKA.](Fig. 4A: Raw data for PKA standard curve (0.007-10 ng/well).)

- **Analytical Characteristics**
  - First-order rate constant of ATP consumption is measured making the assay suitable for all kinases and all types of substrates
  - Variations in ATP and luciferase activity cancel out in the calculations and do not affect assay results
  - Linear range covers over three orders of magnitude
  - \(Z’\) as high as 0.96
  - High kinase activity measured in minutes
  - Low kinase activity detectable by measuring for hours
  - Easy to set up (only one substrate to optimize and wide linear range makes it easy to choose the best kinase level for HTS)
  - All competitive and non-competitive inhibitors detected
  - Kinase BB Kit is a Real-Time Reaction Rate assay of any kinase activity
  - In HTS the light emission is measured twice, which obviates problems with classification resulting from luciferase inhibition
  - In non-HTS further advantages can be gained by reading the plate repeatedly
  - No radioactivity, no antibodies, no conjugates and linear rather than sigmoidal standard curve