

## Quantification of protein kinases and other ATP depletion assays using Kinase RR Kit

Enzymatic ATP depletion assays can be continuously monitored by ATP Reagent SL, if set up as 1<sup>st</sup> order reactions with an ATP concentration well below the  $K_m$  values of both luciferase and the reaction being studied. The logarithm of remaining ATP versus time will then be a straight line. This can be used for HTS of compound libraries to find inhibitors of e.g. protein kinases measuring remaining ATP by the luciferase reaction.

- No interference from luciferase inhibiting compounds or variations in ATP concentration (see Figure 2).
- All library compounds can be classified without follow-up testing.
- Very low ATP depletion by the luciferase reaction (<0.06 % per minute corresponding to  $t_{1/2}=14$  h).
- The kit has been used to monitor ATP depletion reactions by e.g., protein kinases, ATPases and aminoacyl tRNA transferases.
- Microplates (96 or 384 wells) can be used.
- Z' values as high as 0.96 have been obtained.

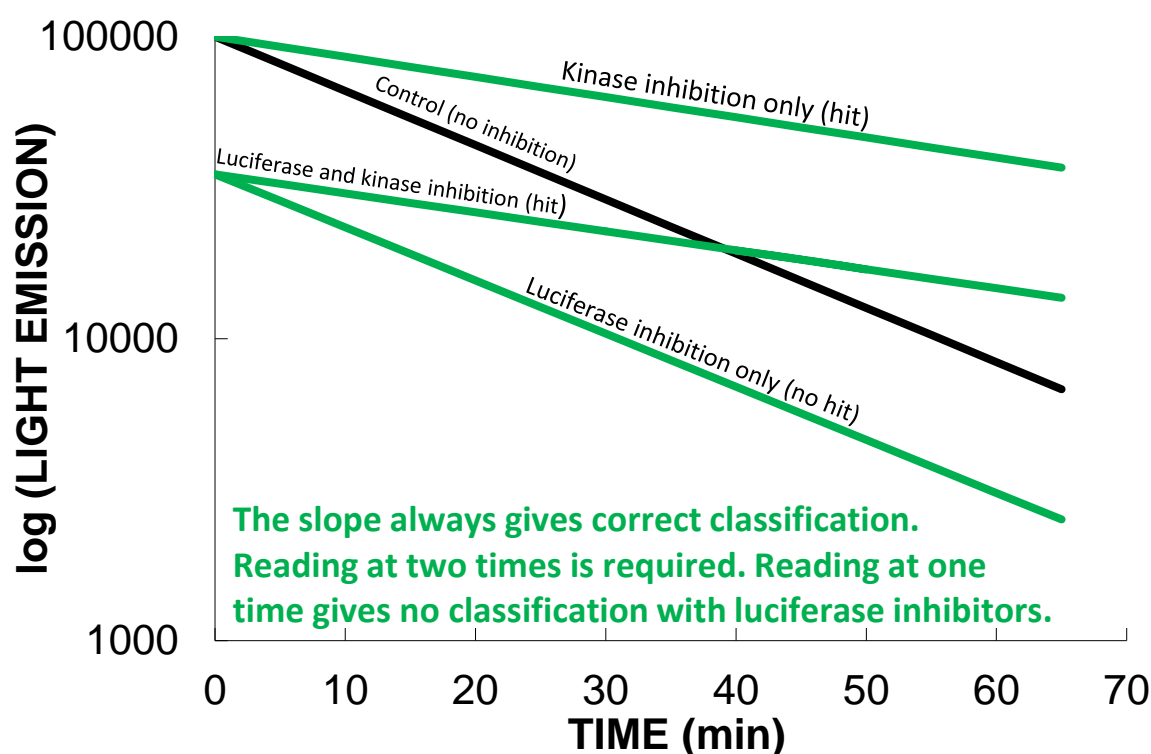


Figure 2: Classification of hit/no hit by time course of light in presence of luciferase inhibitors.

### Examples of applications

1. Measurement of ATP depletion reactions like protein kinase, aminoacyl-tRNA synthetases and ATPases
2. Optimization of substrates and other reaction parameters in ATP depletion reactions
3.  $IC_{50}$  determination for inhibitors of ATP depletion reactions
4. High throughput screening of compound libraries for ATP depletion reactions

References and more details are found in our application notes.