A Novel Bioluminescent HTS Method for Rapid NAD(P)/NAD(P)H Detection

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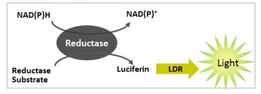
Abstract #256

1. Principle of Bioluminescent NAD(P)/NAD(P)H Detection Technology

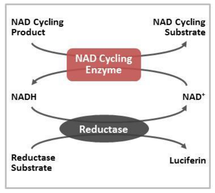
Nicotinamide adenine dinucleotides (NAD+, NADH, NADP+ and NADPH) are fundamental co-factors of cellular energy metabolism. These dinucleotides are essential for macromolecule biosynthesis and the maintenance of the cellular redox potential. Our new rapid, easy-to-use assays for measuring dinucleotides are convenient tool for investigating their role in these processes.

Novel Proluciferin Substrate plus Specific Cycling Enzymes = Three Assays

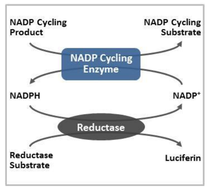
NAD(P)H-Glo™ Detection System- detects NADH and NADPH biochemically



NAD/NADH-Glo™ Assay – detects non-phosphorylated forms in cells

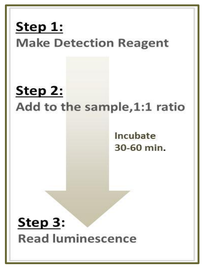


NADP/NADPH-Glo™ Assay – detects phosphorylated forms in cells

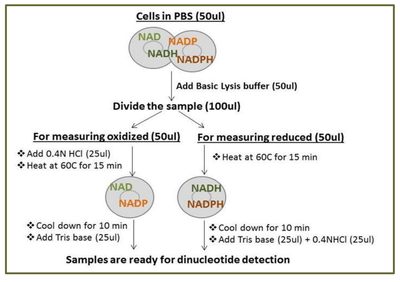


2. Assays are rapid and easy to use: All three use the same protocols but measure different dinucleotides

Rapid in-well, one-step, add & read protocol for total dinucleotide detection



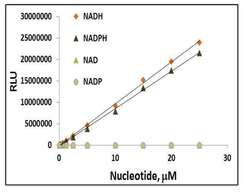
Improved protocol for individual dinucleotide detection (NAD, NADH, NADP, NADPH)



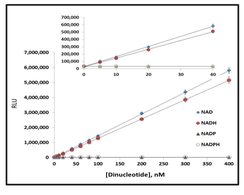
- ✓ Assay sensitivity enables detection of total **NAD+NADH** or **NADP+NADPH** directly in 96/384wells
- ✓ All four dinucleotides are detected from the same starting sample
- ✓ The ratio (**NAD/NADH** or **NADP/NADPH**) is calculated directly from RLU values

3. Low nM sensitivity with maximum S/B>400

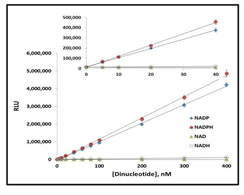
NADH + NADPH



NAD + NADH

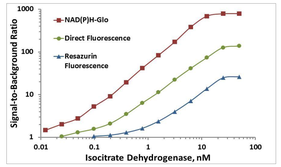


NADP + NADPH



	NAD(P)H-Glo	NAD/NADH-Glo	NADP/NADPH-Glo
Sensitivity (S/B >3)	50nM	4nM	7nM
Linearity	50-25µM	4-500 nM	4-500 nM
Signal Window (S/B)	500	481	430

4. Biochemical Approaches: measuring production reduced or oxidized forms

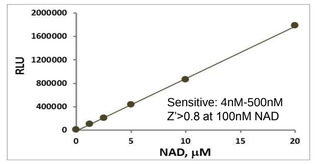


NAD(P)H-Glo Detection System – measure changes in reduced forms in the presence of oxidized forms

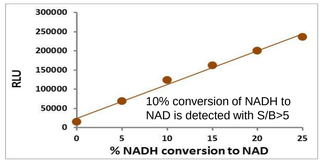
- ✓ Direct detection without signal amplification
- ✓ Might need to stop enzyme reaction before addition of detection reagents

Measuring biochemical activity using **NAD/NADH-Glo** Assay

Enzymes involved in NAD biosynthesis or NAD-dependent signaling reactions (no NADH present)



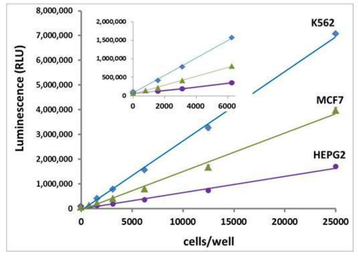
Measuring NADH conversion to NAD (NADH has to be degraded by acids)



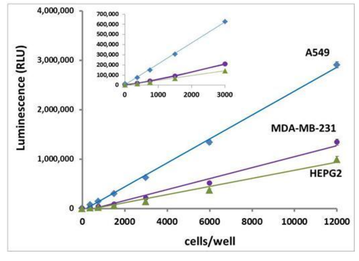
5. Total NAD+NADH or NADP+NADPH are measured rapidly using simple "add and read" protocol

Direct in-well dinucleotide detection using simple add and read protocol

NAD/NADH-Glo™ Assay



NADP/NADPH-Glo™ Assay

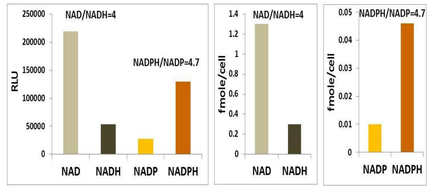


- ✓ To measure NAD+NADH levels the NAD/NADH-Glo reagent is added directly to cells at 1:1 ratio
- ✓ To measure NADP+NADPH levels the NADP/NADPH-Glo reagent is added directly to cells at 1:1 ratio

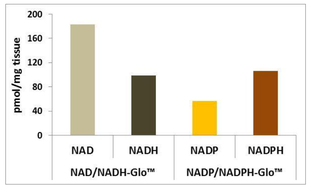
6. Sensitivity of the assays simplify the detection of individual NAD, NADH, NADP, NADPH dinucleotides

- ✓ **All four dinucleotides are detected from a single well of cells in 96- or 384-well plates**
- ✓ **Dinucleotide ratio can be calculated directly from RLU values**
- ✓ **The amount is calculated from calibration curve or from a spike of known dinucleotide amounts**

Determining dinucleotide levels in Jurkat cells

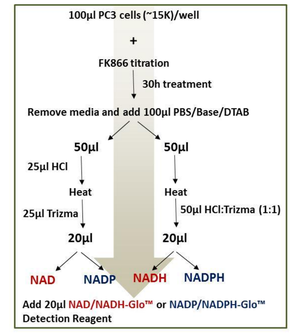


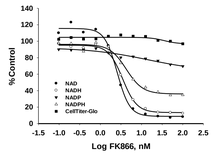
Determining dinucleotide levels in rat liver tissues

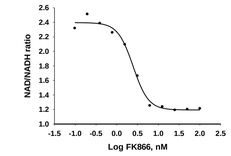


7. Monitoring drug induced changes in cellular NAD, NADH, NADP, NADPH levels

Measuring FK866 effect on individual NAD, NADH, NADP, NADPH levels



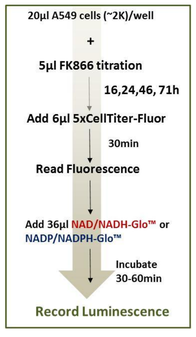


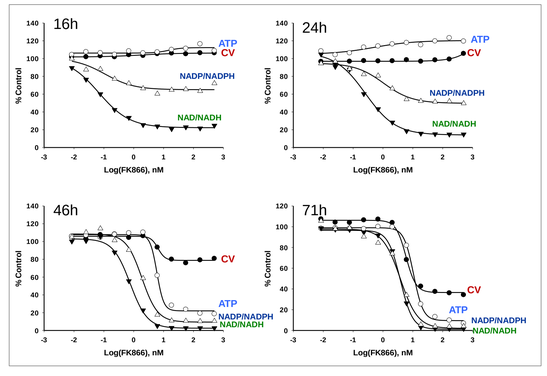


FK866	NAD	NADH	NAD/NADH
IC 50, nM	2.5	3.4	2.5

8. Drug induced changes are detected rapidly without sample processing

Measuring FK866 effect on total NAD+NADH or NADP+NADPH levels

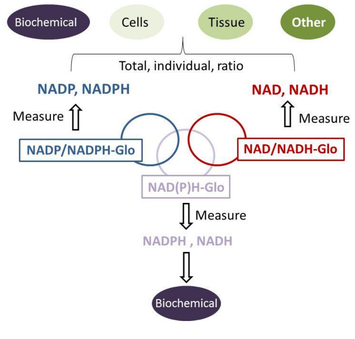




9. Summary: Three new assays for selective detection of NAD, NADH, NADP, NADPH

NAD, NADH, NADP and NADPH serve as important target-independent nodes:

- ✓ They link the metabolic state of cells with energy homeostasis and gene regulation



- ➢ **NAD(P)/NAD(P)H-Glo™** Assays meet the need for rapid and robust measurement of nicotinamide adenine dinucleotides
- ➢ Upstream events that are coupled to **NAD(P)/NAD(P)H** production can be analyzed rapidly with higher precision

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