

# Cell-based metabolic profiling assays for cell therapy

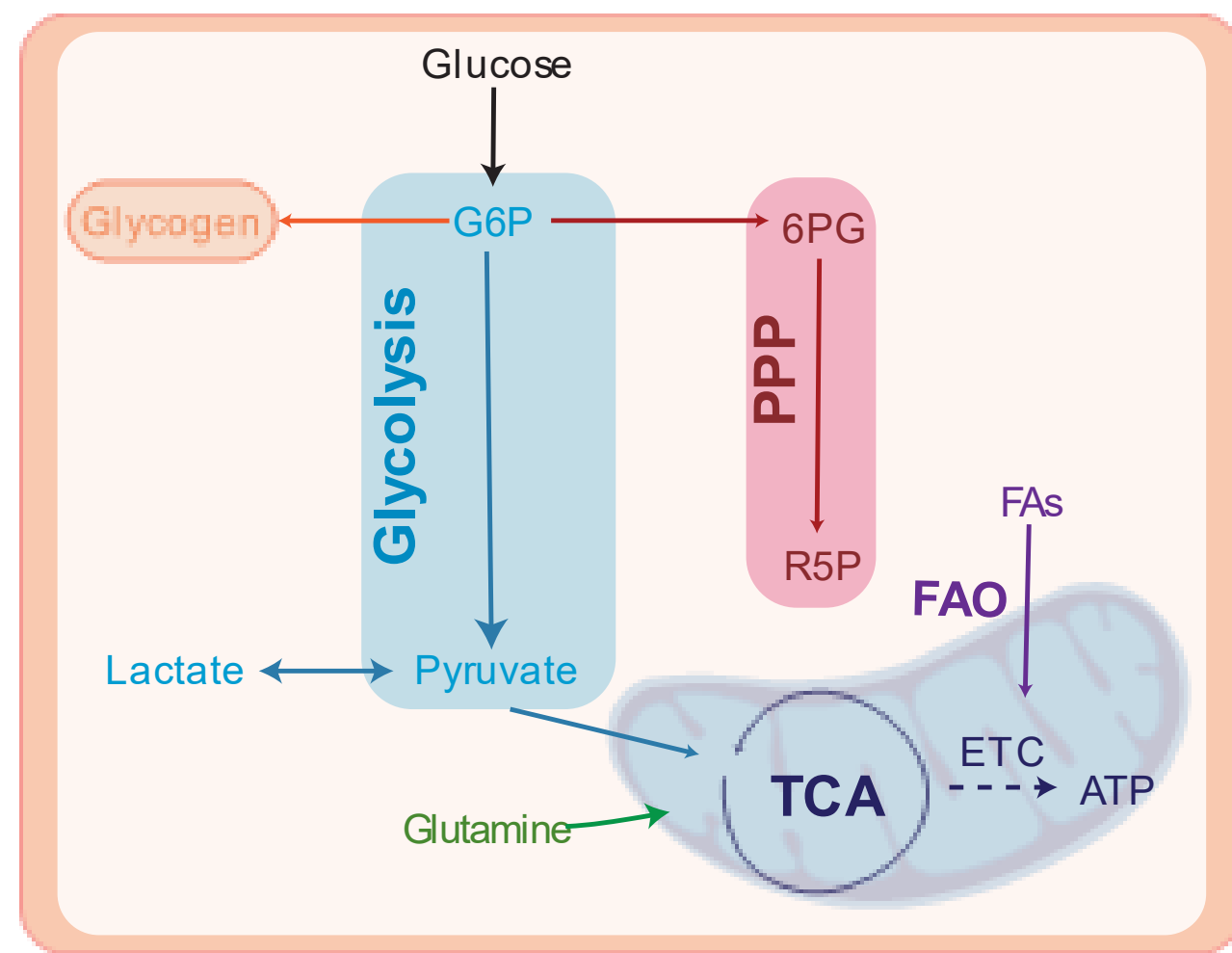


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## 1. Overview

### Bioluminescent assays for metabolic pathway analysis

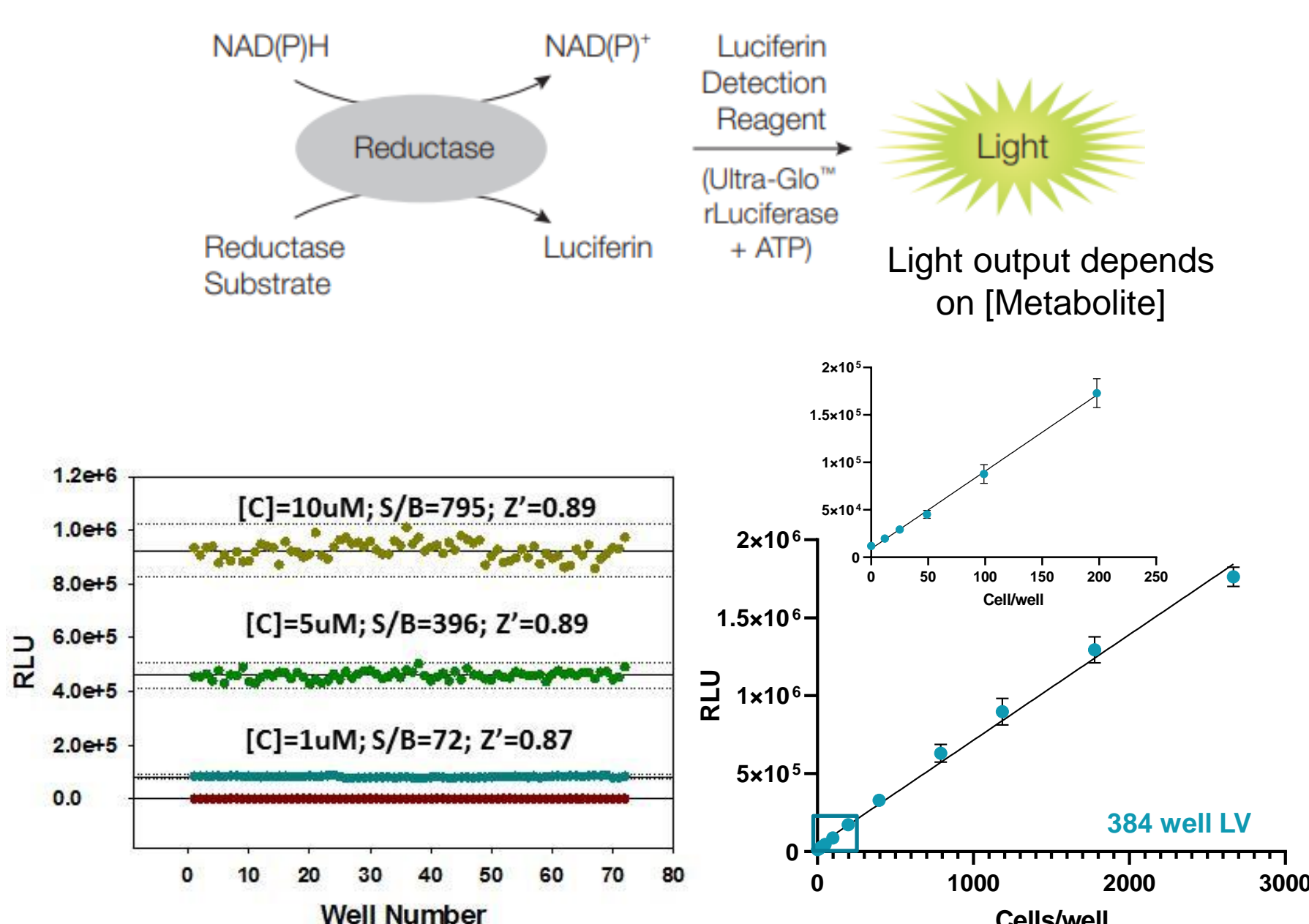


- Pathway Analysis
- Enzyme Activity (DHs)
- Fuel Consumption
- Mitochondrial Function
- Fuel Secretion
- Cell Viability

Fast and easy bioluminescent cell-based assays for monitoring cell health for cell therapies

## 2. Introduction

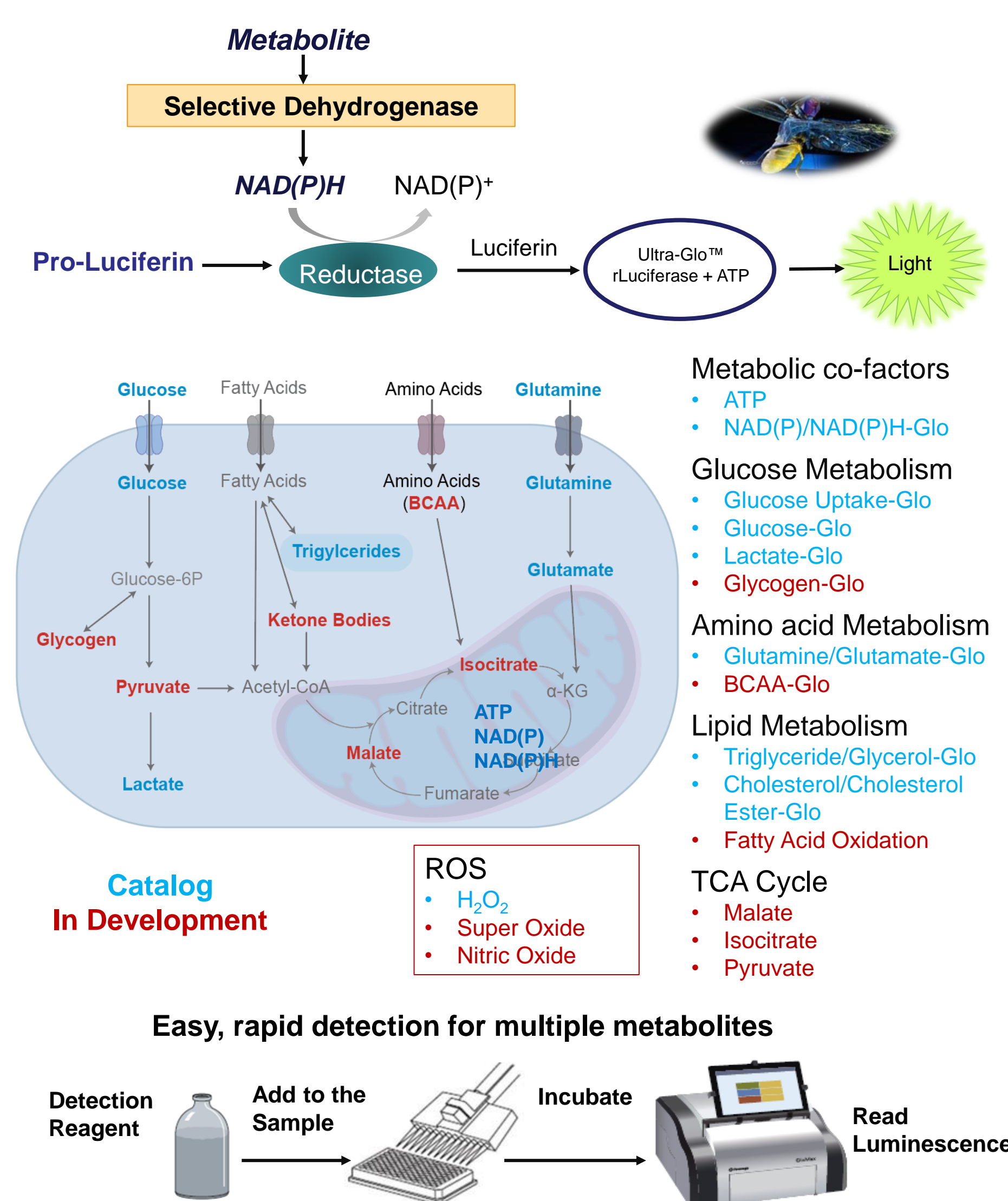
### Bioluminescent NAD(P)H Core Technology



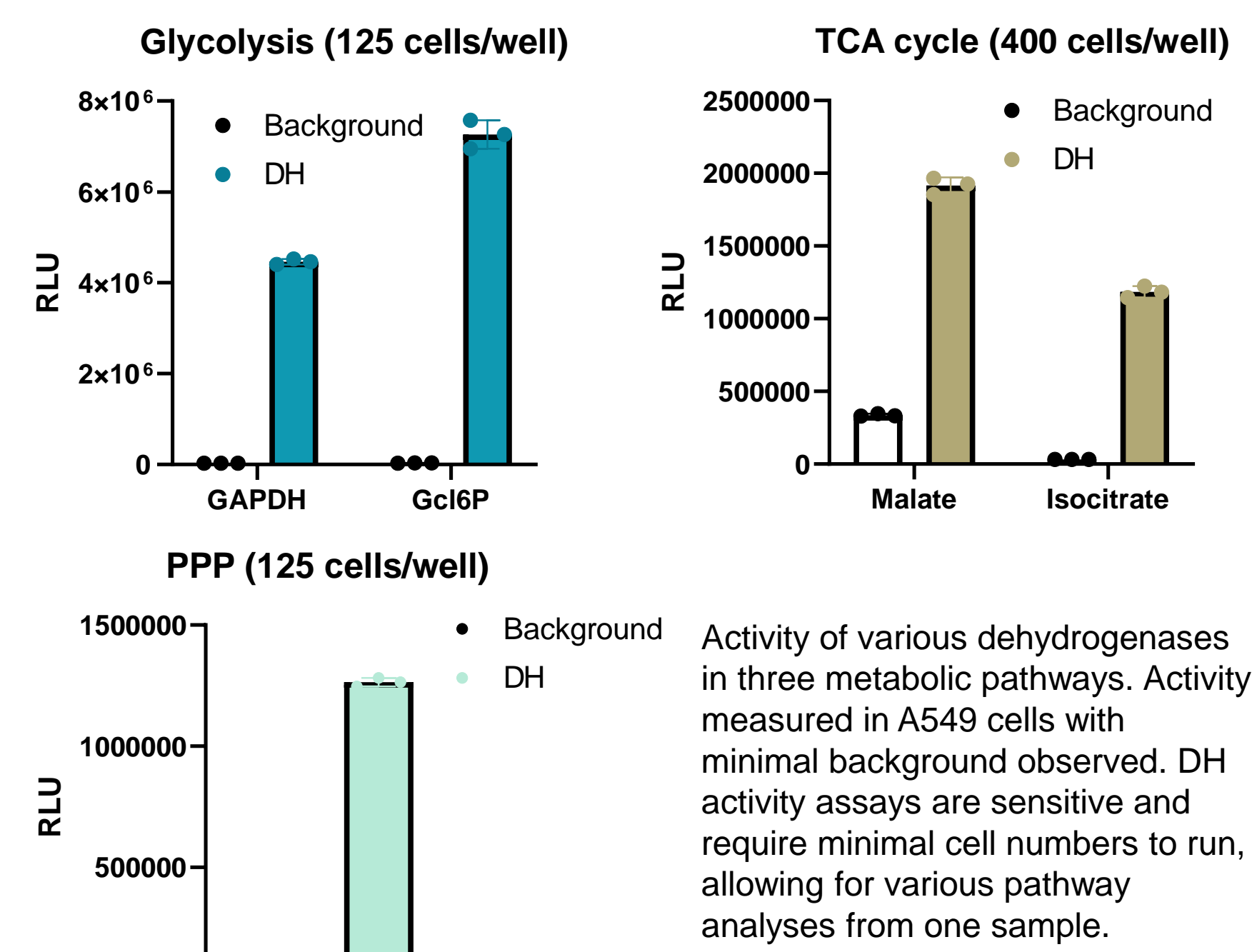
NAD(P)H-Glo is robust assay with high sensitivity and has been used for HTS. Concentration [C], Signal to background (S/B) and Z' are depicted.

Detection of NAD in K562 cells in a 384-well LV plate. NAD is detectable in a linear range from 25,000 cells to 10 cells/well.

## 3. Bioluminescent NAD(P)H-based Metabolite Assays



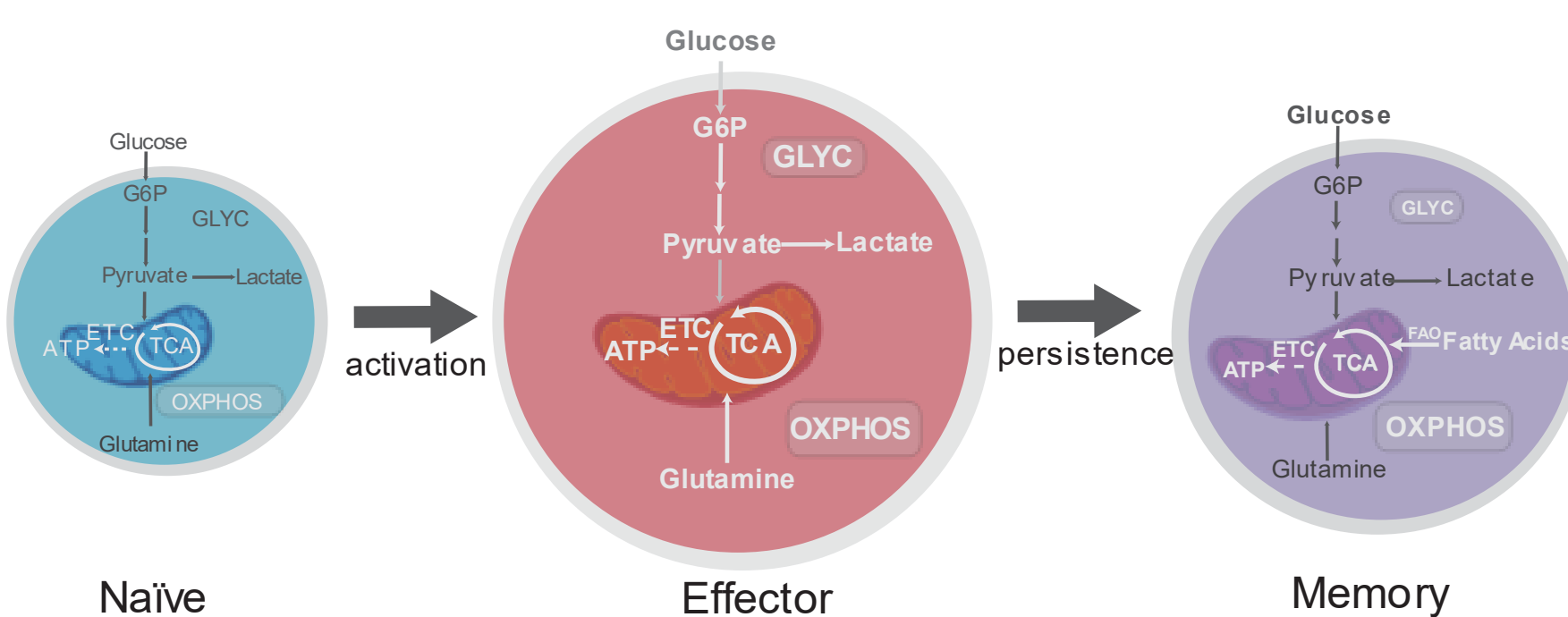
## 4. Monitoring Metabolic Pathways through DHs



Activity of various dehydrogenases in three metabolic pathways. Activity measured in A549 cells with minimal background observed. DH activity assays are sensitive and require minimal cell numbers to run, allowing for various pathway analyses from one sample.

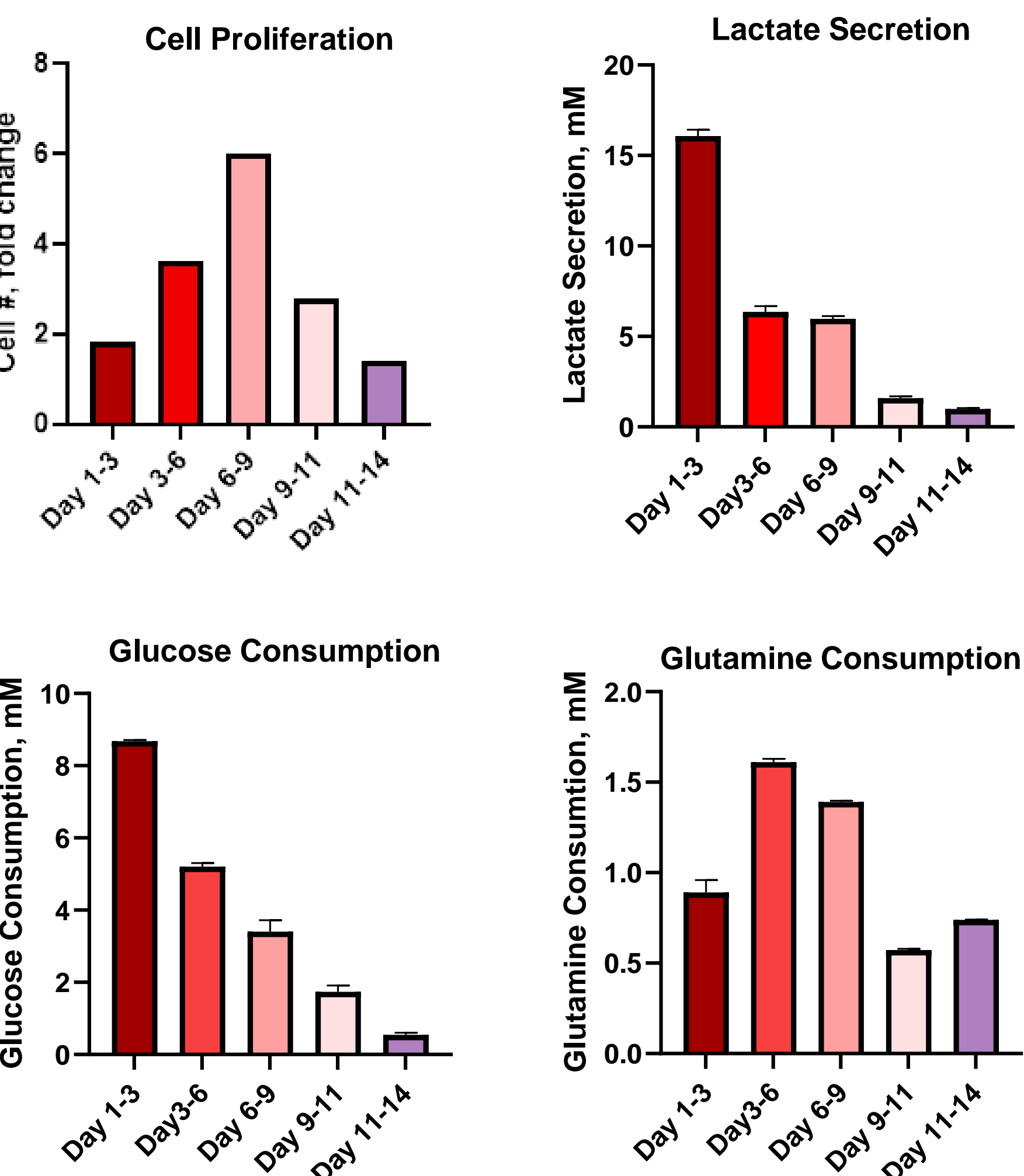
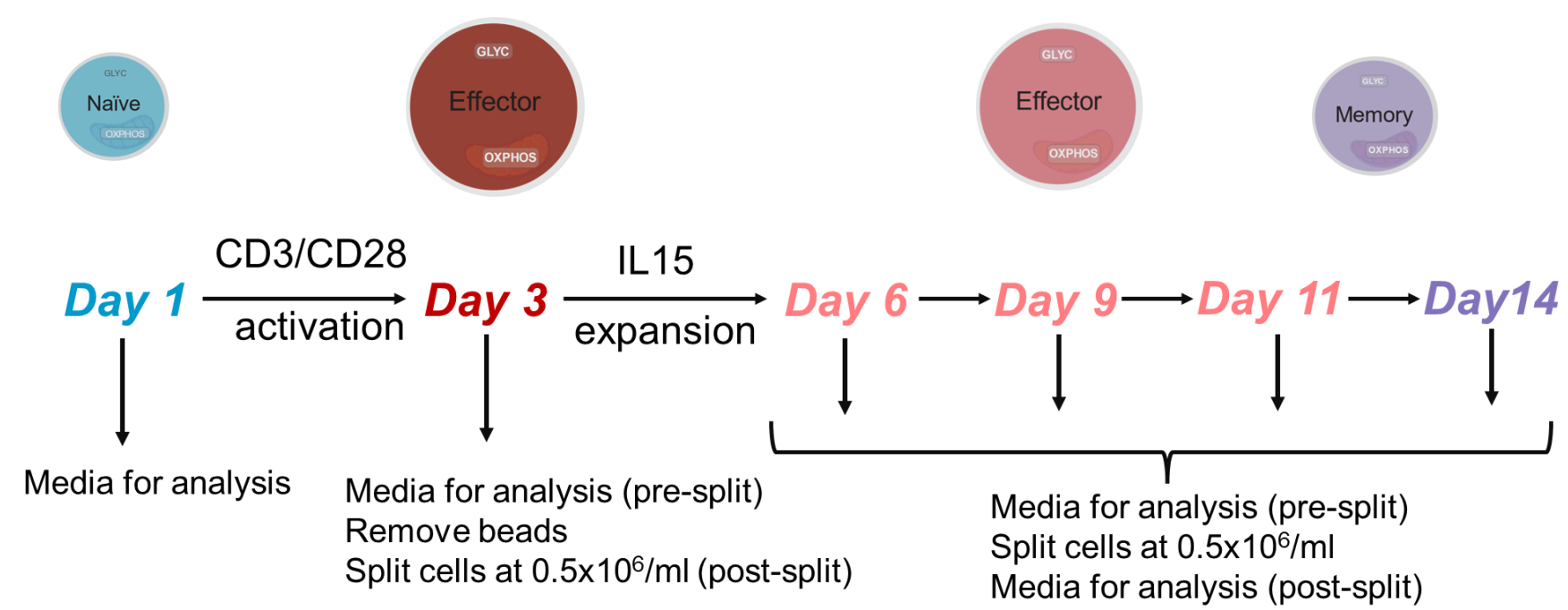
Sensitivity of detection allows for low cell input

## 5. T-cell Metabolism Overview



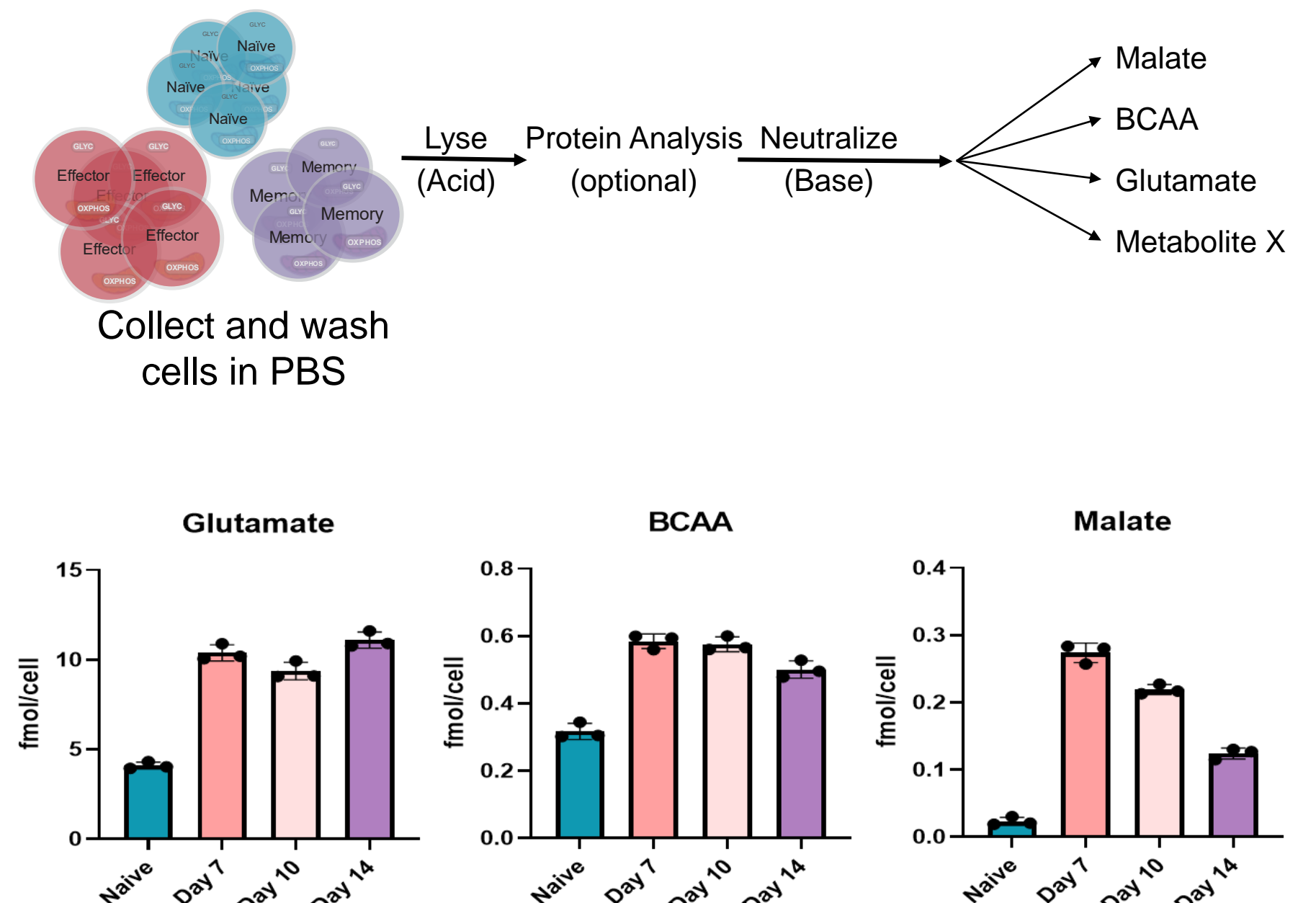
- Metabolically quiescent
- Basal OXPHOS
- Highly metabolically active
- Increased glycolysis and OXPHOS
- Increase fuel uptake
- More quiescent state
- Relies on FAO and OXPHOS

## 6. Activation and Expansion: Changes in Media



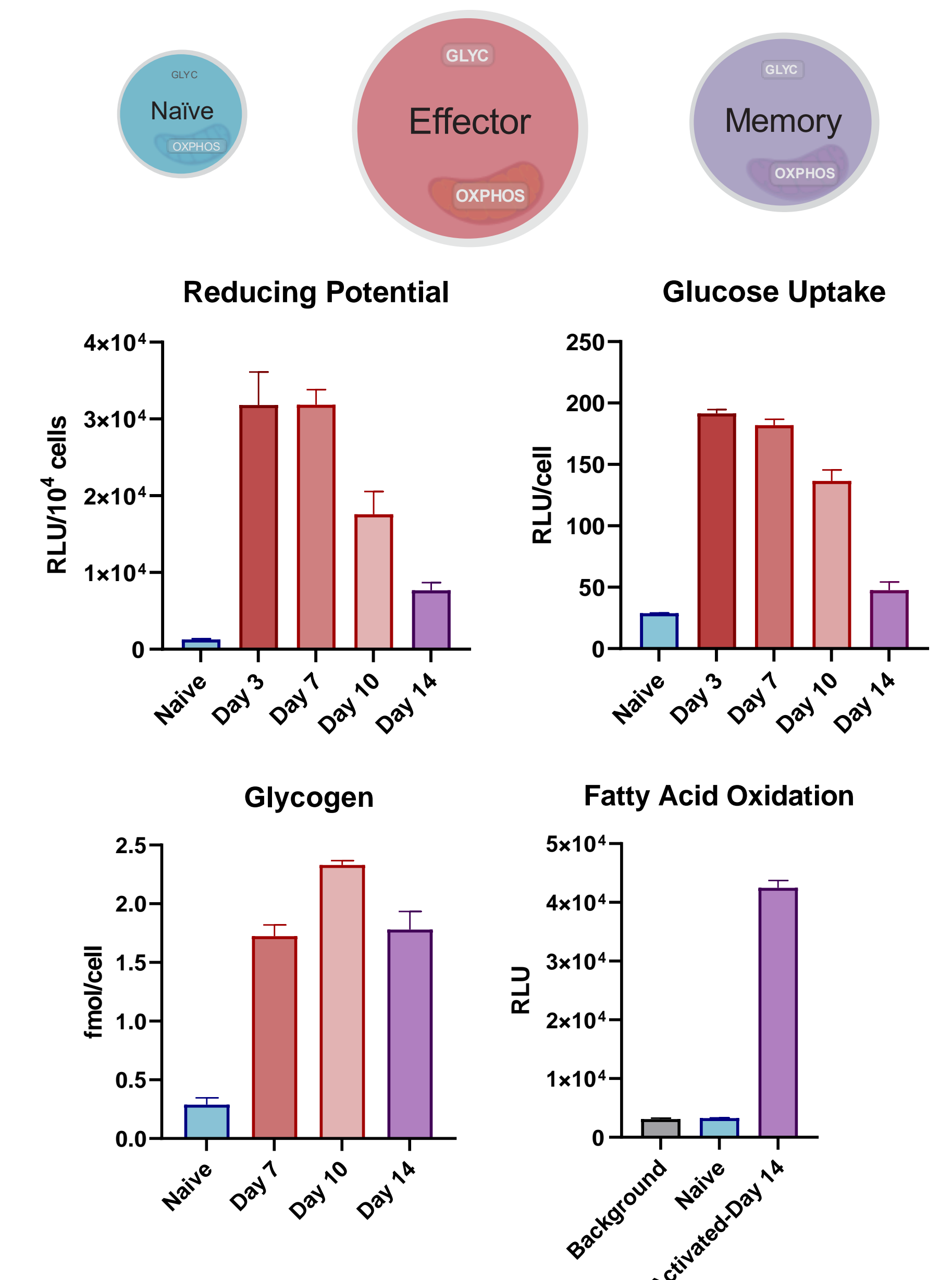
Media metabolite changes vary throughout activation and expansion of T-cells

## 7. T-cell Intracellular Metabolite Detection



Intracellular metabolite levels in naive, activated T-cells (effector) and memory-like cells. Cells were collected and washed with PBS to remove any metabolites present in the media. T-cells were then lysed, neutralized and split into three metabolite specific assays (Glutamate-Glo™, BCAA-Glo™, and Malate-Glo™). Concentrations are depicted as fmole/cell.

## 8. Building Pathway Specific T-Cell Metabolic Profiles



Understanding the metabolic profile of T-cells will aid screening for the most 'fit' cells for cell therapy (i.e., CAR T-cells)

## 9. Outlook

- Exploring bioluminescent metabolic assays for T-cell and CAR T characterization.
- Understanding how metabolic profiles change for different cell types and varying conditions/disease and how this can be leveraged for better cell therapies.

