

# Assuring Optimal Promega Assays Development by Design of Experiment Workflow and Mantis Dispenser

Gediminas Vidugiris<sup>1</sup>, Donna Leippe<sup>1</sup>, Jolanta Vidugiriene<sup>1</sup>, Cristopher Cowan<sup>1</sup>, Kodiak Burke<sup>2</sup>, Paul Ventura<sup>2</sup>

<sup>1</sup>Promega Corporation, 2800 Woods Hollow Rd, Madison, WI, 53711; <sup>2</sup>Formulatrix Inc., 10 DeAngelo Drive, Bedford, MA 01730

Abstract # 1039

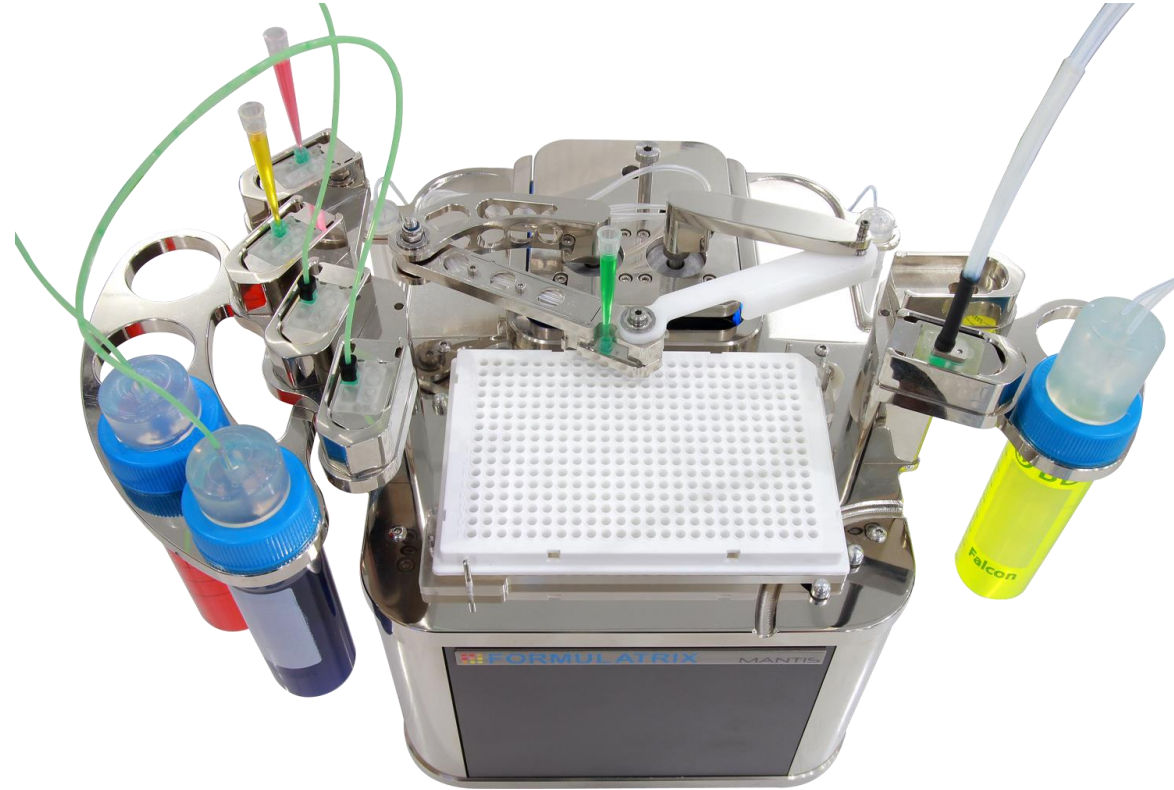


## 1. Introduction

To facilitate assay development we employ a Design of Experiment approach during assay development. Design of Experiments (DoE) offers a systematic way of simultaneously evaluating multiple assay parameters and analyzing the resulting process outputs to optimize assay conditions with a minimum number of experiments.

One of the limitations in taking full advantage of the benefits offered by DoE is the complexity of experimental set up. The Mantis instrument from Formulatrix simplifies DoE setup by providing the unique capabilities of dispensing small volumes of multiple assay components with minimal dead volume into randomly designated wells. Flexibility in the dispensing capabilities and user friendly programming makes this instrument well suited for DoE workflow.

## 2. Mantis - liquid handling instrument from Formulatrix Inc.

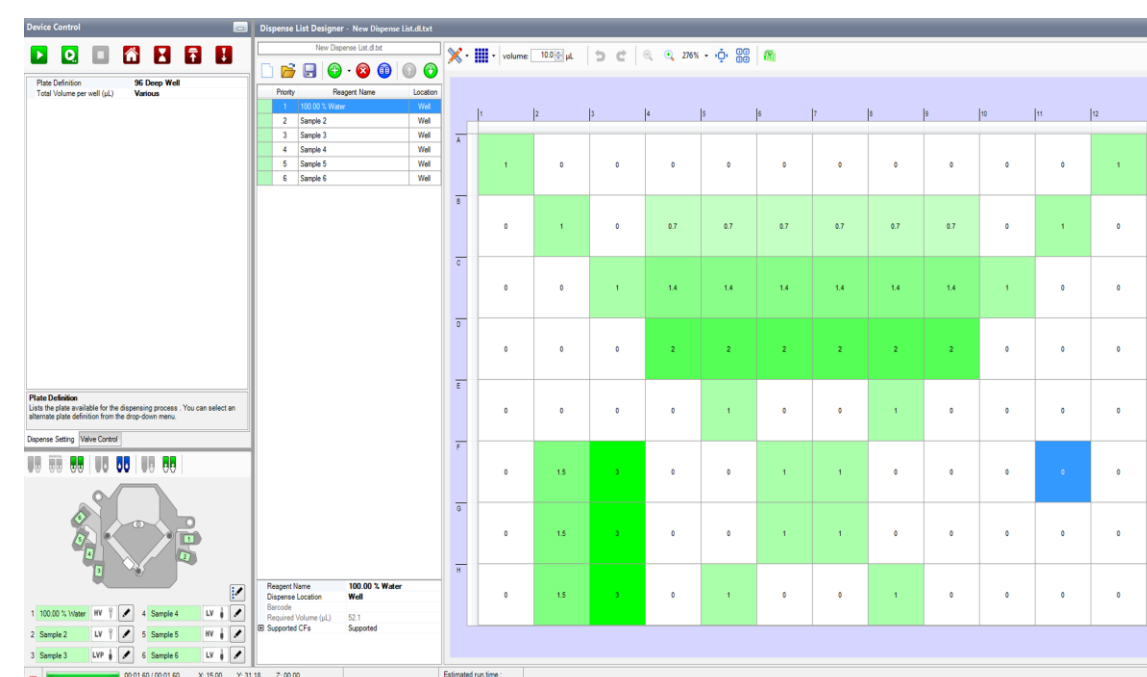


### Mantis advantages:

- Fast, random well access in 96, 384, 1536 well plates
- Dispense volumes 0.1µl – 2 ml or more
- Conserves reagent with only 6 µl dead volume
- Easy to use, intuitive software
- Dual Wash Stations
- Built-in Tube Holders
- Small Footprint
- Solvent compatible

### Available Options:

- Automatic Chip Changer
- Continuous Flow Dispensing

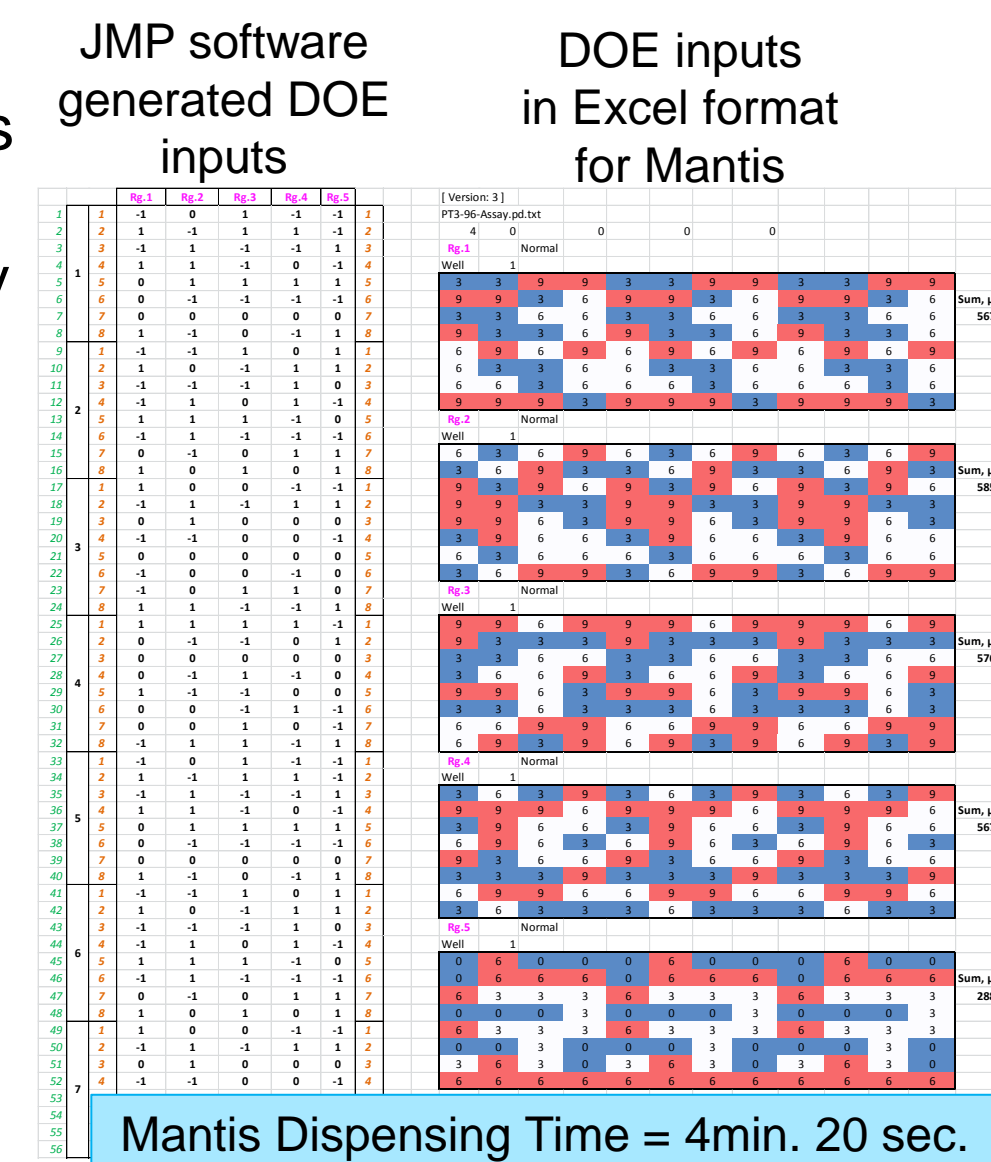


## 3. Design Of Experiment (DoE)

DoE generates statistically designed experiments and associated data analysis that clearly establishes the relationships between the measured parameters (responses) and the process parameters (input factors) being studied.

“Definitive screening design” allows major reduction in the number of experiments: to investigate 5 assay components at 3 concentrations DoE reduces the number of experiments from  $3^5 = 243$  down to 13!).

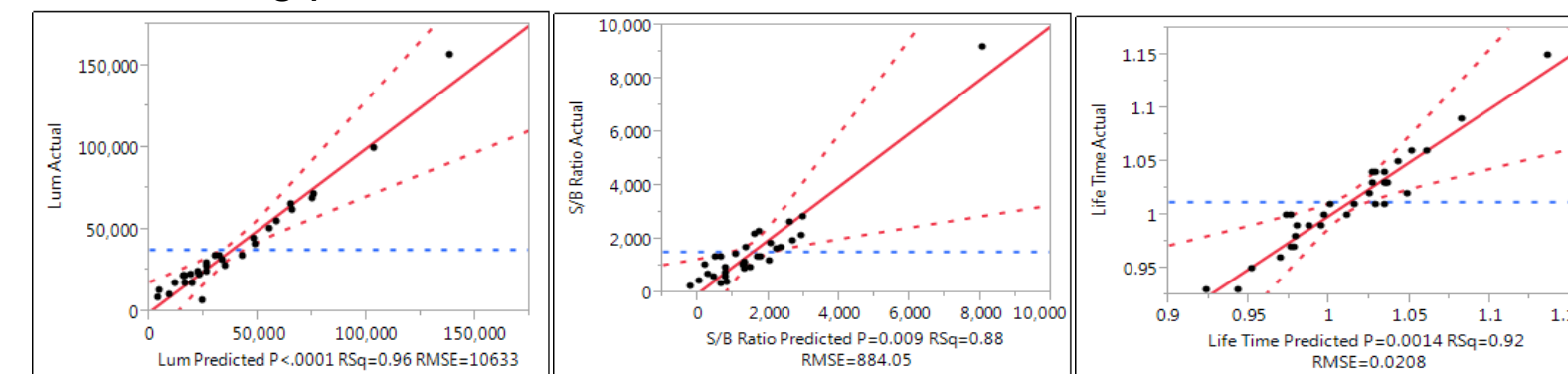
Completely randomized design eliminates possible erroneous conclusions arising from possible flaws caused by: plate edge effects, luminescence reader drifts and liquid dispensing inaccuracies.



## 4. JMP software Fitting and Prediction Plots:

JMP software package used to perform DoE data analysis:

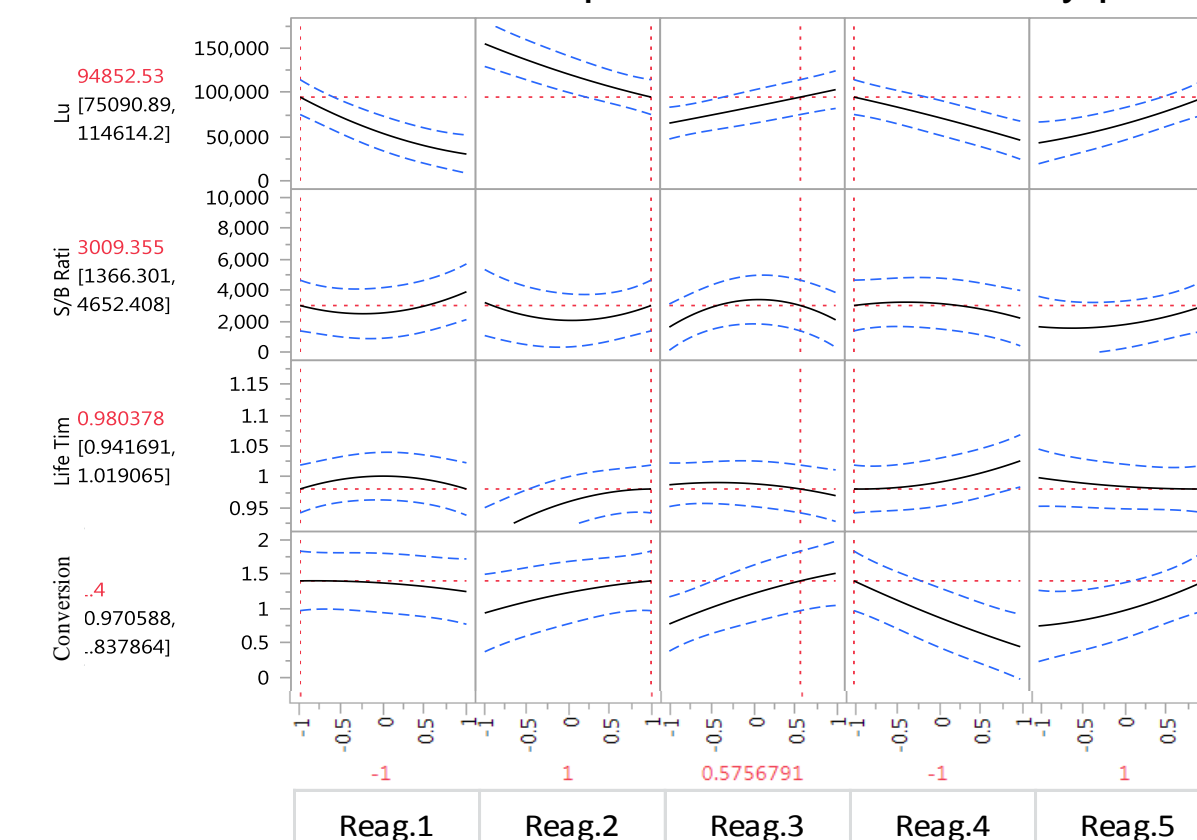
Model fitting plots evaluates confidence of the statistical model :



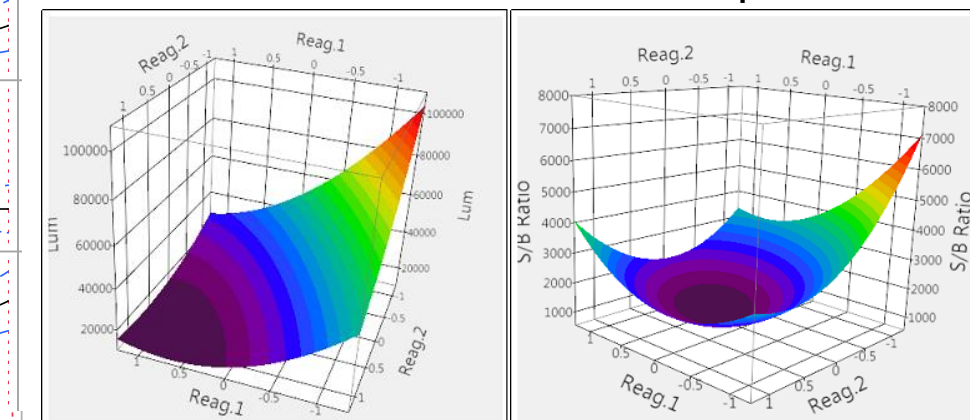
Factors and interactions are evaluated and sorted:

Term	Prob> t
Reag.1	<0.0001*
Reag.4	0.0001*
Reag.5	0.0002*
Reag.2	0.0002*
Reag.3	0.0019*
Reag.1*Reag.5	0.0146*
Reag.1*Reag.3	0.0379*
Reag.1*Reag.4	0.0407*
Reag.1*Reag.2	0.0520
Reag.3*Reag.5	0.0790
Reag.3*Reag.4	0.0832
Reag.4*Reag.5	0.0927
Reag.2*Reag.5	0.1564
Reag.2*Reag.3	0.1693
Reag.5*Reag.5	0.2855
Reag.2*Reag.4	0.3653
Reag.4*Reag.4	0.4214
Reag.3*Reag.4	0.7147
Reag.3*Reag.3	0.9041

Prediction Profiler allows optimization of the assay performance:



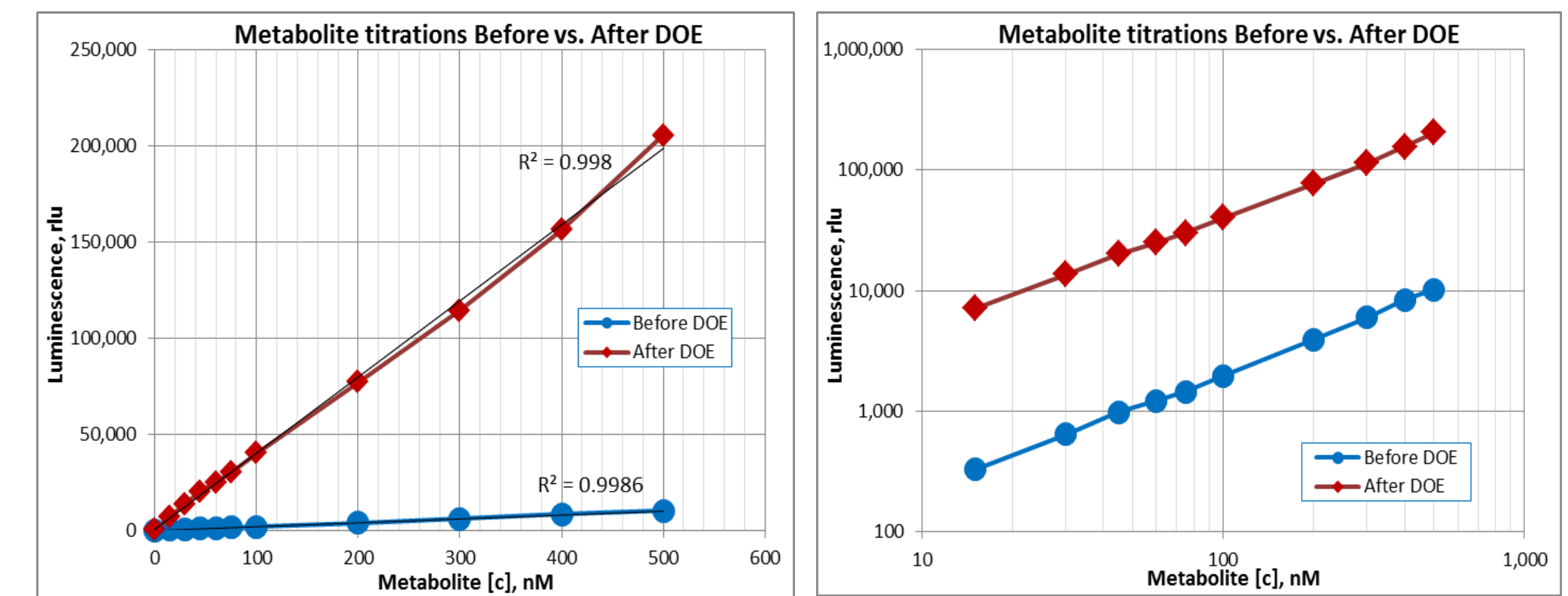
Detailed factors evaluation and optimization:



## 5. Significant Assay Improved after DoE

DoE based assay formulation resulted in significant improvements:

- Luminescence signal increase 20 times
- Signal/Background ratio increased 2 times
- Improvements above achieved without sacrifices in assay lifetime
- Linear luminescence signal response to metabolite concentration
- Wide and linear assay dynamic range



## 6. Conclusions

By implementing DoE and Formulatrix Mantis we quickly achieved substantial assay performance improvements:

- Luminescence signal intensity increased 20 times
- Signal/Background ratio increased 2 times
- Improvements achieved without sacrifices in assay lifetime

Manti is very effective liquid dispenser for DoE experiments:

- Fast, random well access
- Lowest dead volumes required
- Simple to run
- Small footprint

DoE approach in assay development greatly minimized number or the experiments (runs) required to achieve conclusive results. It translates in to great savings on:

- Expenditures on consumables
- Time consumption

