XENOTECH
A BioIVT Company

Data Sheet

rapidSTART™
NADPH Regenerating System

Product ID: K5100
Lot No.: 2110281
Original Expiration Date: 19 July 2022
Extended Expiration Date*: 30 September 2024

Content	*Concentration in Incubation*
NADPH	0.675 mM

*: The expiration date was extended based on ongoing stability testing of the product.

NADPH is added to microsomal incubations to support reactions catalyzed by cytochrome P450, flavin-containing monooxygenases and certain other enzymes. RapidStart provides the required NADPH for these reactions by generating NADPH *in situ* through an enzymatic reaction catalyzed by glucose-6-phosphate.

Vial contents: 0.1 mL of 100 mM NADP
0.1 mL of 500 mM Glucose-6-phosphate
0.1 mL of 100 units/mL Glucose-6-phosphate dehydrogenase
In 20 mM potassium phosphate buffer, pH 7.4

Preparation procedures:

1. Determine the desired initial concentration using the dilution chart provided (page 2).
2. Add the appropriate amount of high purity water for desired concentration.
3. Vortex vial until all three pellets have fully dissolved.
4. NADPH has been generated and the working solution is ready for use.

Stability of activated NADPH regenerating system:
At 2-8°C: 48 hours
At room temperature: 12 hours

Do not refreeze.

The total number of incubations that can be performed is dependent on the assay design.

Suggested Use:

Store at -20°C or below

Intended for in vitro *use only.*

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In a typical microsomal incubation, the recommended concentration of the NADPH-regenerating system consists of 1 mM NADP(H), 5 mM glucose-6-phosphate and 1 Unit/mL of glucose-6-phosphate dehydrogenase. These concentrations can be achieved by adding 0.70 mL of high purity water to the frozen components in the RapidStart vial, vortexing, and then adding the activated RapidStart solution to the incubation mixture in a 1:10 (v/v) dilution to initiate metabolic reactions.

RapidStart is designed as a flexible prepackaged NADPH regenerating system to accommodate various experimental designs. Depending on the amount of high purity water added to the system, RapidStart generates NADPH with a concentration of ~4-80 mM.

RapidStart Dilution Table

High Purity Water Added (mL)	NADP (mM)		G6P (mM)		G6PDH (units/mL)		Total Volume (mL)	Expected NADPH Concentration, As Prepared (mM)
	As Prepared	Final Incubation 1:10 (v/v)	As Prepared	Final Incubation 1:10 (v/v)	As Prepared	Final Incubation 1:10 (v/v)		
0.10	25.00	2.50	125.00	12.500	25.00	2.500	0.40	~17-22
0.20	20.00	2.00	100.00	10.000	20.00	2.000	0.50	~14-18
0.30	16.67	1.67	83.33	8.333	16.67	1.667	0.60	~10-13
0.40	14.29	1.43	71.43	7.143	14.29	1.429	0.70	~8.8-12.8
0.50	12.50	1.25	62.50	6.250	12.50	1.250	0.80	~10.0-11.2
0.60	11.11	1.11	55.56	5.556	11.11	1.111	0.90	~7.8-10
0.70*	**10.00**	**1.00**	**50.00**	**5.000**	**10.00**	**1.000**	**1.00**	**~7.0-9.0**
0.80	9.09	0.91	45.45	4.545	9.09	0.909	1.10	~6.4-8.2
0.90	8.33	0.83	41.67	4.167	8.33	0.833	1.20	~5.8-7.5
1.00	7.69	0.77	38.46	3.846	7.69	0.769	1.30	~5.4-6.9
1.10	7.14	0.71	35.71	3.571	7.14	0.714	1.40	~5.0-6.4
1.20	6.67	0.67	33.33	3.333	6.67	0.667	1.50	~4.7-6.0
1.30	6.25	0.63	31.25	3.125	6.25	0.625	1.60	~4.4-5.6
1.40	5.88	0.59	29.41	2.941	5.88	0.588	1.70	~4.1-5.3
1.50	5.56	0.56	27.78	2.778	5.56	0.556	1.80	~3.9-5.0
1.60	5.26	0.53	26.32	2.632	5.26	0.526	1.90	~3.7-4.7
1.70	5.00	0.50	25.00	2.500	5.00	0.500	2.00	~3.5-4.5

* Denotes XenoTech's recommended concentration.

NADPH Concentration Determination:

Although NADP and NADPH both absorb at 260 nm, only NADPH absorbs strongly at 340 nm. Consequently, the reduction of NADP to NADPH is associated with an increase in absorbance at ~340 nm. This increase in absorbance at ~340 nm can be used to determine the concentration of NADPH.

To verify the concentration of NADPH generated, a spectrophotometer is used to determine absorbance at ~340 nm (338-341nm is acceptable). Dispense 995 µL of high purity water into a sample well and 1000 µL into a reference well. Add 5 µL of prepared RapidStart to the sample well and record the absorbance at 340 nm. The concentration of NADPH in an incubation can be determined using the following equation.

$$\frac{Absorbance}{\varepsilon} \times \frac{Volume\ of\ NADPH\ added\ to\ incubation\ mixture\ (\mu L)}{Incubation\ volume\ (\mu L)} \times \frac{Total\ volume\ in\ sample\ well\ (\mu L)}{Volume\ of\ NADPH\ added\ to\ well\ (\mu L)} = Concentration\ in\ incubation\ \ (mM)$$

where **ε** = extinction coefficient of NADPH = 6.22 mM-1cm-1 (which indicates that a 1 mM solution of NADPH would have an absorbance value of 6.22 in a 1 cm pathlength).