

## p-Nitroaniline (pNA)

CATALOG #: 1078-100

AMOUNT: 100 mg

LOT #: \_\_\_\_\_

### STRUCTURE:

FORMULA:  $C_6H_6N_2O_2$

MOL. WEIGHT: 138.1

FORM: Gold powder

PURITY: >99% by GC



### DESCRIPTION:

The free form of pNA can be used as a colorimetric marker and standard in colorimetric caspase activity assays.

### QUANTIFICATION OF CASPASE ACTIVITY:

1. Generate a pNA Calibration Curve:

- Dissolve 13.81 mg pNA in 1 ml DMSO. Dilute the 100 mM pNA stock solution in DMSO to make 0, 0.5, 1, 2 and 4 mM stock solutions.
- To 5  $\mu$ l of each stock solution, add 95  $\mu$ l of Cell Lysis Buffer to give these final concentrations:

5 $\mu$ l	0 mM pNA + 95 $\mu$ l of Buffer	=	0 nmole pNA
5 $\mu$ l	0.5 mM pNA + 95 $\mu$ l of Buffer	=	2.5 nmole pNA (25 $\mu$ M)
5 $\mu$ l	1.0 mM pNA + 95 $\mu$ l of Buffer	=	5.0 nmole pNA (50 $\mu$ M)
5 $\mu$ l	2.0 mM pNA + 95 $\mu$ l of Buffer	=	10 nmole pNA (100 $\mu$ M)
5 $\mu$ l	4.0 mM pNA + 95 $\mu$ l of Buffer	=	20 nmole pNA (200 $\mu$ M)

- Measure the five dilutions with a spectrophotometer and prepare a calibration curve with  $x = \text{nmol pNA}$  and  $y = \text{O.D. Units (ODU)}$ .

**Sample Results** (read at 405 nm; your results may vary):

nmol pNA	ODU
0	0.001
2.5	0.071
5.0	0.149
10	0.254
20	0.508

- Use the slope ( $\Delta\text{ODU}/\Delta\text{nmol pNA}$ ) of this curve to calculate units of caspase activity with the following formula:

$$\text{Units Caspase} = (\Delta\text{ODU}/\text{hr}) \times \frac{1}{\text{curve slope}}$$

( $\Delta\text{ODU}/\text{hr}$  = the difference in ODU between  $T_0$  and  $T_1$ )

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