

PRODUCT: p-Nitroaniline (pNA)

CATALOG #: 1078-100
AMOUNT: 100 mg
LOT #: _____
FORMULA: C₆H₆N₂O₂
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:

- 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 µl of each stock solution, add 95 µl of Cell Lysis Buffer to give these final concentrations:

5 µl	0 mM pNA + 95 µl of Buffer	=	0 nmole pNA
5 µl	0.5 mM pNA + 95 µl of Buffer	=	2.5 nmole pNA (25 µM)
5 µl	1.0 mM pNA + 95 µl of Buffer	=	5.0 nmole pNA (50 µM)
5 µl	2.0 mM pNA + 95 µl of Buffer	=	10 nmole pNA (100 µM)
5 µl	4.0 mM pNA + 95 µl of Buffer	=	20 nmole pNA (200 µM)
 - Measure the five dilutions with a spectrophotometer and prepare a calibration curve with x = nmol pNA and y = O.D. Units (ODU).

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

<u>nmol pNA</u>	<u>ODU</u>
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 T₀ and T₁)

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- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Set
- Apoptosis siRNA Vectors

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- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
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