

PRODUCT: *p*-Nitroaniline (*p*NA)

CATALOG #:	1078-100
AMOUNT:	100 mg
LOT #:	_____
FORMULA:	C ₆ H ₆ N ₂ O ₂
MOL. WEIGHT:	138.1
FORM:	Gold powder
PURITY:	>99% by GC

DESCRIPTION:

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

QUANTIFICATION OF CASPASE ACTIVITY:

1. Generate a *p*NA Calibration Curve:
 - a. Dissolve 13.81 mg *p*NA in 1 ml DMSO. Dilute the 100 mM *p*NA stock solution in DMSO to make 0, 0.5, 1, 2 and 4 mM stock solutions.
 - b. To 5 µl of each stock solution, add 95 µl of Cell Lysis Buffer to give these final concentrations:

5 µl	0 mM <i>p</i> NA + 95 µl of Buffer	=	0 nmole <i>p</i> NA
5 µl	0.5 mM <i>p</i> NA + 95 µl of Buffer	=	2.5 nmole <i>p</i> NA (25 µM)
5 µl	1.0 mM <i>p</i> NA + 95 µl of Buffer	=	5.0 nmole <i>p</i> NA (50 µM)
5 µl	2.0 mM <i>p</i> NA + 95 µl of Buffer	=	10 nmole <i>p</i> NA (100 µM)
5 µl	4.0 mM <i>p</i> NA + 95 µl of Buffer	=	20 nmole <i>p</i> NA (200 µM)
 - c. Measure the five dilutions with a spectrophotometer and prepare a calibration curve with $x = \text{nmol } p\text{NA}$ and $y = \text{O.D. Units (ODU)}$.

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

<u>nmol <i>p</i>NA</u>	<u>ODU</u>
0	0.001
2.5	0.071
5.0	0.149
10	0.254

2. Use the slope ($\Delta \text{ODU}/\Delta \text{nmol } p\text{NA}$) 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)

RELATED PRODUCTS:

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2. Histone Deacetylase Fluorometric & Colorimetric Assay Kits
3. Calpain Activity Assay Kit
4. Apoptotic Cell Isolation Kit
5. MitoCapture™ Mitochondrial Apoptosis Detection Kit
6. Mitochondria/Cytosol Fractionation Kit
7. Nuclear/Cytosol Fractionation Kit
8. Cytochrome c Releasing Apoptosis Assay Kit
9. ApoSENSOR™ ATP Determination Kit & ADP/ATP Ratio Assay Kit
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13. CaspSCREEN™ Flow Cytometric Caspase Assay Kit
14. CaspGLOW™ Active Caspase Staining Kits
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