

Instruction Manual

Catalog Number	PK-CA577-1078-100												
Description	The free form of pNA can be used as a colorimetric marker and standard in colorimetric caspase activity assays.												
Quantity	100 mg												
Sequence / Molecular Weight / Molecular Formula	138,1 Da; C ₆ H ₆ N ₂ O ₂												
Purity	>99% by GC												
Appearance / Formulation / Solubility	Gold powder												
Storage & Stability	Store at -20°C.												
Applications	<p>Quantification of Caspase activity:</p> <p>1. Generate a pNA Calibration Curve:</p> <p>a. Dissolve 13.81 µg pNA in 1 ml DMSO. Dilute the 100 µM pNA stock solution in DMSO to make 0, 0.5, 1, 2 and 4 mM stock solutions.</p> <p>b. To 5 µl of each stock solution, add 95 µl of Cell Lysis Bfer (Cat. # PK-CA577-1067-100, -400) to give these final concentrations:</p> <p>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)</p> <p>c. Measure the five dilutions with a spectrophotometer and prepare a calibration curve with x = nmol pNA and y = O.D. Units (ODU).</p> <p>Sample Results (read at 405 nm; your results may vary):</p> <table border="1"> <thead> <tr> <th>nmol pNA</th> <th>ODU</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>0.001</td> </tr> <tr> <td>2.5</td> <td>0.071</td> </tr> <tr> <td>5.0</td> <td>0.149</td> </tr> <tr> <td>10</td> <td>0.254</td> </tr> <tr> <td>20</td> <td>0.508</td> </tr> </tbody> </table> <p>2. Use the slope ($\Delta\text{ODU}/\Delta\text{nmol pNA}$) of this curve to calculate units of caspase activity with the following formula: Units Caspase = ($\Delta\text{ODU}/\text{hr}$) x 1/curve slope ($\Delta\text{ODU}/\text{hr}$ = the difference in ODU between T0 and T1)</p>	nmol pNA	ODU	0	0.001	2.5	0.071	5.0	0.149	10	0.254	20	0.508
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References	NA												
Caution	NA												

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