

# CASPASE-3 COLORIMETRIC ASSAY LAYOUT SHEET

for use with Assay Designs' Catalog No. 907-013

## • REAGENT PREPARATION:

- To make Caspase-3 Reaction Buffer (1x): Add 2 mL of Caspase-3 Reaction Buffer Concentrate to 18 mL of DI H<sub>2</sub>O and mix well.
- To make Active Caspase-3 Reaction Buffer: Using 20 mL of diluted Caspase-3 Reaction Buffer (1x), add 1 mL of this buffer to one DTT vial. Vortex and transfer the entire contents to the remaining 19 mL of Caspase-3 Reaction Buffer. Rinse the DTT vial by adding 1 mL of this Buffer to the DTT vial, vortex and return contents to the now Active Caspase-3 Reaction Buffer.
- Caspase-3 Substrate, 1x: Add 10 mLs of Active Caspase-3 Reaction Buffer to a clean container. From this volume, remove 100  $\mu$ L of Active Caspase-3 Reaction Buffer and add 100  $\mu$ L of the Caspase-3 Substrate Concentrate.

## TABLE FOR MAKING STANDARDS:

Std.	Active Reaction Buffer Vol. ( $\mu$ L)	Vol. Added ( $\mu$ L)	Caspase-3 Conc. (Units/mL)
1	250	Lyoph., Stock	1,000
2	125	125, Std. 1	500
3	125	125, Std. 2	250
4	125	125, Std. 3	125
5	125	125, Std. 4	62.5

\*Keep Standards in ice bath and use within 1 hour of preparation

## Caspase-3 Reconstituted Standard

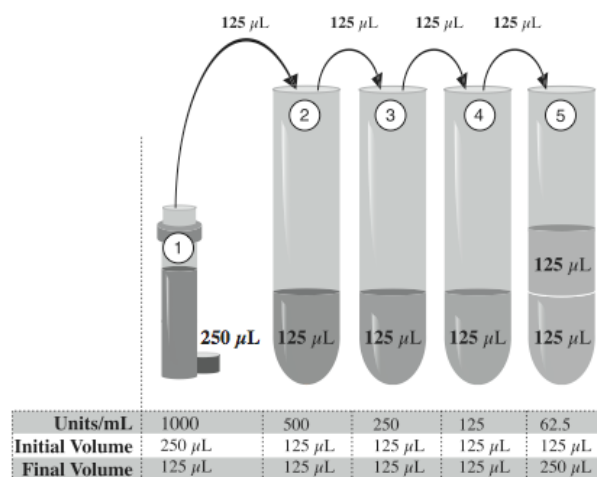


Figure 2

## ASSAY PROTOCOL FLOW CHART:

Well I.D.:	Blank A1, B1	Stds. C1 – D2	pNA E2 – F2	Samples G2 - H12
Standard and/or Sample	---	50 $\mu$ L	---	50 $\mu$ L
Active Reaction Buffer, 1x	50 $\mu$ L	---	---	---
p-nitroaniline	---	---	125 $\mu$ L	---
Substrate	75 $\mu$ L	75 $\mu$ L	---	75 $\mu$ L
Incubate 3 hrs. at 37°C, sealed	---	---	---	---
Optional: Add 1N HCl Stop	10 $\mu$ L	10 $\mu$ L	10 $\mu$ L	10 $\mu$ L
Read @ 450 nm				

• **CASPASE-3 PLATE LAYOUT:**

A1 Blank	A2 Std 4	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	B2 Std 4	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 Std. 1	C2 Std 5	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 Std. 1	D2 Std 5	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 Std 2	E2 pNA	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Std 2	F2 pNA	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Std 3	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Std 3	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kit Lot No. \_\_\_\_\_ Exp. Date \_\_\_\_\_ Date \_\_\_\_\_ Tech. \_\_\_\_\_

Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_ Notes: \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 \_\_\_\_\_  
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