

CASPASE-3 FLUOROMETRIC ASSAY LAYOUT SHEET

for use with Assay Designs' Catalog No. 907-014

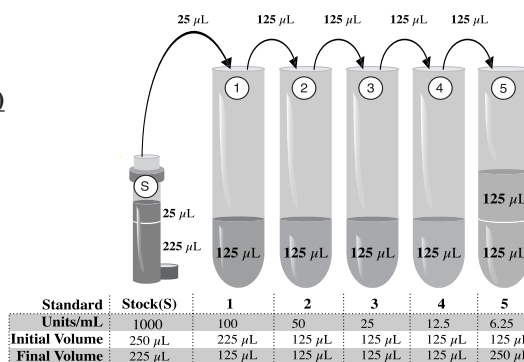
• REAGENT PREPARATION:

- To make Caspase-3 Reaction Buffer (1x): Add 2 mL of Caspase-3 Reaction Buffer Concentrate to 18 mL of DI H₂O 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 20 µL of Active Caspase-3 Reaction Buffer and add 20 µL of the Caspase-3 Substrate Concentrate. Stable at 4°C for 3 months.

• TABLE FOR MAKING STANDARDS:

Std.	Active Reaction Buffer Vol. (µL)	Vol. Added (µL)	Caspase-3 Conc. (Units/mL)
Stock	250	Lyoph., Stock	1,000
1	225	25, Stock	100
2	125	125, Std. 1	50
3	125	125, Std. 2	25
4	125	125, Std. 3	12.5
5	125	125, Std. 4	6.25

Caspase-3 Reconstituted Standard



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

• ASSAY PROTOCOL FLOW CHART:

Well I.D.:	Blank A1, B1	Stds. C1 - D2	AMC E2 - F2	Samples G2 - H12
Standard and/or Sample	---	50 µL	---	50 µL
Active Reaction Buffer, 1x	50 µL	---	---	---
AMC Calibrator	---	---	200 µL	---
Substrate	150 µL	150 µL	---	150 µL
Incub. 3 hrs. at 37°C, sealed	---	---	---	---
Optional: Add 1N HCl Stop	20 µL	20 µL	20 µL	20 µL

Read on fluorometer 360 nm excitation / 440 nm emission

• **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 AMC	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Std. 2	F2 AMC	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. _____

1st Incub.: Start Time _____ Temp. _____ Notes: _____

End Time _____ Temp. _____

2nd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____