

cAMP Complete ASSAY LAYOUT SHEET

For use with Enzo Life Sciences Catalog Nos. ADI-900-163 and ADI-901-163

• **DILUTION TABLES FOR MAKING STANDARDS 1-5:**

Non-Acetylated Version:

Std.	*Std. Dil. Vol. (µL)	Vol. Added (µL)	cAMP Conc. (pmol/mL)
1	900	100, Stock	200
2	750	250, Std. 1	50
3	750	250, Std. 2	12.5
4	750	250, Std. 3	3.12
5	750	250, Std. 4	0.78

Acetylated Version:

Std.	*Std. Dil. Vol. (µL)	Vol. Added (µL)	cAMP Conc. (pmol/mL)
1	990	10, Stock	20
2	750	250, Std. 1	5
3	750	250, Std. 2	1.25
4	750	250, Std. 3	0.312
5	750	250, Std. 4	0.078

* Three options are available for the sample diluent. Assay Buffer 2, 0.1M HCl, or non-conditioned culture media. Please see the instruction manual for further information.

• **ACETYLATION PROCEDURE (Optional):**

- 1) Prepare Acetylating Reagent by mixing 0.5 mL of Acetic Anhydride with 1 mL of Triethylamine.
- 2) Add 10 µL of the Acetylating Reagent to 200 µL of standard (including 0 and NSB) or sample. Vortex immediately.
- 3) Assay the acetylated standards and samples within 30 minutes.

• **ASSAY PROTOCOL FLOW CHART:**

Well I.D.:	Blank A1, B1	TA C1, D1	NSB E1, F1	Zero Std. (Bo) G1, H1	Stds. A2 - B3	Samples C3 - H12
**Neutralizing Reagent	---	---	50 µL	50 µL	50 µL	50 µL
Standard Diluent	---	---	100 µL	100 µL	---	---
Assay Buffer 2	---	---	50 µL	---	---	---
Std. and/or Sample	---	---	---	---	100 µL	100 µL
Conjugate	---	---	50 µL	50 µL	50 µL	50 µL
Antibody	---	---	---	50 µL	50 µL	50 µL
Incub. 2 hours @ RT,	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 3 x 200µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	5 µL	---	---	---	---
Substrate	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL
Incub. 1 hour @ RT	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	50 µL	50 µL	50 µL	50 µL	50 µL	50 µL

** Neutralizing Reagent is only added if 0.1M HCl is used for preparation of standards and samples. Do not use with the other diluent options.

• **DIRECT cAMP PLATE LAYOUT:**

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