Direct cAMP Assay Layout Sheet

- **Table of Dilutions for Making Standards:**

Acetylated Version

Std.	0.1M HCl Vol. (µL)	Vol. Added (µL)	cAMP Conc. (pmol/mL)
1		_____, stock (5000 pmol/mL)	
2		_____, Std. 1	
3		_____, Std. 2	
4		_____, Std. 3	
5		_____, Std. 4	
6		_____, Std. 5	
7		_____, Std. 6	
0			

- **Acetylation Procedure:**

_____ µL of Acetic Anhydride with _____ µL of Triethylamine (1:2)
_____ µL of Acetylating Reagent added to each standard
_____ µL of Acetylating Reagent added to each sample

- **Assay Flow Chart**

Well I.D.	Blank	TA	NSB	Bo.	Stds.	Samples
Neutralizing Reagent	---	---	50 µL	50 µL	50 µL	50 µL
0.1 M HCl	---	---	150 µL*	100 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, shaking	⇒ ⇒ ⇒	⇒ ⇒ ⇒	⇒ ⇒ ⇒	⇒ ⇒ ⇒	⇒ ⇒ ⇒	⇒ ⇒ ⇒
Asp. & Wash 3× 200 µL	⇒ ⇒ ⇒	⇒ ⇒ ⇒	⇒ ⇒ ⇒	⇒ ⇒ ⇒	⇒ ⇒ ⇒	⇒ ⇒ ⇒
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

*This 0.1 M HCl with acetylating reagent.

Direct cAMP Plate Layout:

A1 Bo	A2 Bo	A3 Blank	A4 Blank	A5	A6	A7	A8	A9	A10	A11	A12
B1 Std 1	B2 Std 1	B3 TA	B4 TA	B5	B6	B7	B8	B9	B10	B11	B12
C1 Std 2	C2 Std 2	C3 NSB	C4 NSB	C5	C6	C7	C8	C9	C10	C11	C12
D1 Std 3	D2 Std 3	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 Std 4	E2 Std 4	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Std 5	F2 Std 5	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Std 6	G2 Std 6	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Std 7	H2 Std 7	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kit Lot No. _____ Exp. Date _____ Date _____ Tech. _____

1st Incub: Start Time _____ Temp. _____ Note: _____
End Time _____ Temp. _____ _____

2nd Incub: Start Time _____ Temp. _____ _____
End Time _____ Temp. _____ _____