DIRECT cGMP ASSAY LAYOUT SHEET

for use with Assay Designs' Catalog Nos. 900-014 and 901-014 (formerly 90014 and 90114)

• DILUTION TABLES FOR MAKING STANDARDS 1-5:

Non-Acetylated Version:

Std.	0.1M HCl Vol. (µL)	Vol. Added (µL)	cGMP Conc. (pmol/mL)
1	900	100, Stock	500
2	800	200, Std. 1	100
3	800	200, Std. 2	20
4	800	200, Std. 3	4
5	800	200, Std. 4	0.8

Acetylated Version:

Std.	0.1M HCl Vol. (µL)	Vol. Added (µL)	cGMP Conc. (pmol/mL)
1	990	10, Stock	50
2	800	200, Std. 1	10
3	800	200, Std. 2	2
4	800	200, Std. 3	0.4
5	800	200, Std. 4	0.08

• ACETYLATION PROCEDURE:

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 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. G1, H1	Stds. A2 - B3	Samples C3 - H12
Neutralizing Reagent	---	---	50 µL	50 µL	50 µL	50 µL
0.1M 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 or 18-24 hours @ 4˚C, sealed						
Asp. & Wash 3 x 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

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• **DIRECT cGMP 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. _____ _____