



HT Direct Cyclic AMP

Enzyme Immunoassay Kit

Catalog # 2501-096-K

96 Well Kit

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

Trevigen, Inc.



Please
Recycle

HT Direct Cyclic AMP Enzyme Immunoassay Kit

Catalog # 2501-096-K

96 Well Kit

Table of Contents

Page Number

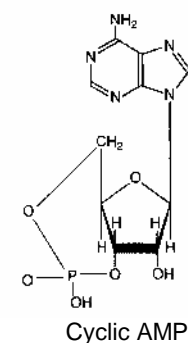
I. Description	2
II. Introduction	2
III. Precautions	3
IV. Materials Supplied	3
V. Storage	3
VI. Materials Required but Not Supplied	4
VII. Sample Preparation	4
VIII. Procedural Notes	5
IX. Reagent Preparation	5
X. Assay Procedure	6
XI. Calculation of Results	7
XII. Typical Results	8
XIII. Typical Standard Curves and Quality Control Parameters	8
XIV. Performance Characteristics	9
XV. Sample Dilution Recommendations	10
XVI. Assay Layout	11
XVII. References	13
XVIII. Related Products Available from Trevigen	13

I. Description

Trevigen's HT Direct cyclic AMP EIA kit is a competitive immunoassay for the quantitative determination of cyclic AMP in samples treated with 0.1M HCl. The kit uses a polyclonal antibody to cAMP to bind, in a competitive manner, the cAMP in the standard or sample or an alkaline phosphatase molecule which has cAMP covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated read on a microplate reader at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of cAMP in either standards or samples. The measured optical density is used to calculate the concentration of cAMP. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard¹ or Tijssen².

II. Introduction

Adenosine 3', 5'-cyclic monophosphate (cyclic AMP; cAMP) is one of the most important "second messengers" involved as a modulator of physiological processes³⁻⁷. cAMP is also involved in regulating neuronal, glandular, cardiovascular, immune and other functions and actions⁸⁻¹¹. A number of hormones are known to activate cAMP through the action of the enzyme adenylate cyclase which converts ATP to cAMP. These hormones include a variety of anterior pituitary peptide hormones such as corticotropin (ACTH), glucagon, calcitonin, thyroid stimulating hormone (TSH) and luteinizing hormone (LH). cAMP has been shown to be involved in the cardiovascular, nervous system, and immune mechanisms, cell growth and differentiation, and general metabolism¹²⁻¹⁴. There remains considerable interest in the measurement of intracellular cAMP in tissues and cell cultures, and this may help to provide an understanding of the physiology and pathology of many disease states.



III. Precautions

FOR RESEARCH USE ONLY. **Not** for use in diagnostic procedures.

1. Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
2. Some solutions supplied in this kit are caustic: care should be taken with their use.
3. The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (> 10 mM) such as EDTA and EGTA.
4. We test this kit's performance with a variety of samples. However, it is possible that high levels of interfering substances may cause variation in assay results.
5. The cyclic AMP Standard provided, Catalog # 2500-096-05, is supplied in ethanolic buffer at a pH optimized to maintain cAMP integrity. Care should be taken in handling this material because of the known and unknown effects of cAMP.

CAUTION: Some components of this kit contain chemicals that are lachrymators, corrosive and flammable. Use with caution and wear suitable protection. Refer to the Material Safety Data Sheets available on request.

IV. Materials Supplied

Catalog Number	Component	Size	Description
2500-096-P	Goat anti-Rabbit IgG Microtiter Plate	96 well plate	A plate using break-apart strips coated with goat antibody to rabbit IgG.
2501-096-01	cAMP Direct Conjugate	6 ml	A blue solution of alkaline phosphatase conjugated with cAMP.
2500-096-02	cAMP EIA Antibody	6 ml	A yellow solution of a rabbit polyclonal antibody to cAMP.
2501-096-02	0.1M HCl	30 ml	0.1M hydrochloric acid in water
2501-096-03	Neutralizing Reagent	6 ml	Alkaline solution for neutralization of 0.1M HCl
2500-096-04	Wash Buffer Concentrate	30 ml	Tris buffered saline containing detergents.
2500-096-05	Cyclic AMP Standard	0.5 ml	A solution of 2,000 pmol/ml cAMP
2500-096-06	p-Npp Substrate	20 ml	A solution of p-nitrophenyl phosphate in buffer. Ready to use.
2501-096-04	Stop Solution	6 ml	A solution of trisodium phosphate in water
2500-096-08	Triethylamine	2 ml	Acetylation reagent
2500-096-09	Acetic Anhydride	1 ml	Acetylation reagent

V. Storage

All components of this kit are stable at 4°C until the kit's expiration date.

VI. Materials Required but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 μ l and 1,000 μ l.
3. Repeater pipets for dispensing 50 μ l and 200 μ l.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

VII. Sample Preparation

Trevigen's Direct cAMP EIA is compatible with samples that have been treated with hydrochloric acid to stop endogenous phosphodiesterase activity. Samples in this matrix can be measured directly without evaporation or further treatment. Please refer to the Sample Dilution Recommendations on page 12 for details of suggested dilutions. However, the end user must verify that the recommended dilutions are appropriate for their samples. We have provided reagents to acetylate samples and standards for samples with very low levels of cAMP.

Tissue samples frozen in liquid nitrogen should be ground to a fine powder under liquid nitrogen in a stainless steel mortar. After the liquid nitrogen has evaporated, weigh the frozen tissue and homogenize in 10 volumes of 0.1M HCl. Centrifuge at > 600 \times g at room temperature. The samples can then be diluted in the 0.1M HCl provided for the assay.

Cells grown in tissue culture media can be treated with 0.1M HCl after first removing the media. Incubate for 10 minutes and visually inspect the cells to verify cell lysis. If adequate lysis has not occurred, incubate for a further 10 minutes and inspect. Centrifuge at 600 \times g at room temperature, then use the supernatant directly in the assay. Cell or tissue lysis can be enhanced by adding 0.1% to 1% Triton X-100 to the 0.1M HCl prior to use. When used in this concentration range, the detergent will not interfere with acetylation or the binding portion of the assay, however there will be a modest increase in the optical density. Samples containing Triton should be evaluated against a cAMP standards containing the same concentration of Triton X-100 for the most accurate determination. Cyclic AMP in the media can be measured after treating 1 ml of the supernatant media with 10 μ l concentrated hydrochloric acid. Centrifuge at > 600 \times g at room temperature. The supernatants can then be used directly in the assay. Note: RPMI-1640 media contains high concentrations (>350 pmol/ml) of endogenous cAMP and residual media on the cells will effect the measurement of intracellular cAMP levels.

VIII. Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or polypropylene tubes; avoid polystyrene.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
8. **Care must be taken to minimize contamination by endogenous alkaline phosphatase.** Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
9. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**

IX. Reagent Preparation

1. **Wash Buffer:** Prepare the Wash Buffer (Cat# 2500-096-04) by diluting 5 ml of the supplied concentrate with 95 ml of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.
2. **cAMP Standard—Non-Acetylated Version:** Allow the 2,000 pmol/ml cAMP standard solution (Cat# 2500-096-05) to warm to room temperature. Label five 12 × 75 mm glass tubes #1 through #5. Pipet 900 µl 0.1M HCl (Cat# 2501-096-02) into tube #1 and 750 µl of 0.1M HCl into tubes #2 - #5. Add 100 µl of the 2,000 pmol/ml cAMP standard to tube #1. Vortex thoroughly. Transfer 250 µl of tube #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #5. *The concentration of cAMP in tubes #1 through #5 will be 200, 50, 12.5, 3.12 and 0.78 pmol/ml, respectively. See Direct cAMP Assay Layout Sheet for dilution details. Diluted standards should be used within 60 minutes of preparation.*
3. **Acetylation Reagent:** Prepare the Acetylating Reagent by adding 0.5 ml of Acetic Anhydride (Cat# 2500-096-09) to 1 ml Triethylamine (Cat# 2500-096-08). Use the prepared reagent within 60 minutes of preparation.
4. **cAMP Standard—Acetylated Version:** Allow the 2,000 pmol/ml cAMP standard solution to warm to room temperature. Label five 12 × 75 mm glass or 1.5 ml polypropylene tubes #1 through #5. Pipet 990 µl 0.1M HCl into tube #1 and 750 µl of 0.1M HCl into tubes #2 – #5. Add 10 µl of the 2,000 pmol/ml standard to tube #1. Vortex thoroughly. Transfer 250 µl of tube #1 to tube #2 and vortex thoroughly. Continue in this way for tubes #3 through #5.

The concentration of cAMP in tubes #1 through #5 will be 20, 5, 1.25, 0.312 and 0.078 pmol/ml, respectively. See Direct cAMP Assay Layout Sheet for dilution details.

Acetylate all standards and samples by adding 10 µl of the Acetylating Reagent for each 200 µl of standard or sample. Add the reagent directly to the samples and vortex for 2 seconds immediately after the addition. To a 12 × 75 mm glass or 1.5 ml polypropylene tube (labeled as the Zero Standard/NSB tube) add 1 ml 0.1M HCl and 50 µl of the Acetylating Reagent. Failure to acetylate the NSB and Zero standard will result in inaccurate B/Bo values. Use the acetylated standards or samples within 30 minutes of preparation.

X. Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening. All standards and samples should be run in duplicate. If Acetylated Version of the kit is to be run, prepare the standards, samples, and Zero Standard/NSB Tube as described in Step IX.4 above.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the Ziploc bag. Store unused wells at 4°C.
 2. Pipet 50 µl of the Neutralizing Reagent into each well, except the Total Activity (TA) and Blank wells.
 3. Pipet 100 µl of 0.1M HCl into the NSB and the B0 (0 pmol/ml Standard) wells.
 4. Pipet 100 µl of Standards #1 through #5 into the appropriate wells.
 5. Pipet 100 µl of the Samples into the appropriate wells.
 6. Pipet 50 µl of 0.1M HCl into the NSB wells.
 7. Pipet 50 µl of the blue Conjugate (Cat# 2501-096-01) into each well except the TA and Blank wells.
 8. Pipet 50 µl of the yellow Antibody (Cat# 2500-096-02) into each well, except the Blank, TA and NSB wells.
- NOTE: Every well used should be **Green** in color except the NSB wells which should be **Blue**. The Blank and TA wells are empty at this point and have no color.*
9. Incubate the plate at room temperature for 2 hours on a plate shaker at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.
 10. Empty the contents of the wells and wash by adding 200 µl of wash solution to every well. Repeat the wash 2 more times for a total of 3 washes. Tap the

plate between washes onto a dry lint free paper towel to remove any remaining wash buffer.

11. Add 5 µl of the blue Conjugate to the TA wells.
12. Add 200 µl of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.
13. Add 50 µl of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

XI. Calculation of Results

Several options are available for the calculation of the concentration of cAMP in the samples. We recommend that the data be handled by an immunoassay software package utilizing a weighted 4 parameter logistic curve fitting program. If this type of data reduction software is not readily available, the concentration of cAMP can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

$$\text{Average Net OD} = \text{Average Bound OD} - \text{Average NSB OD}$$

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Using Logit-Log paper plot Percent Bound (B/Bo) versus concentration of cAMP for the standards. Approximate a straight line through the points. The concentration of cAMP in the unknowns can be determined by interpolation.

XII. Typical Results

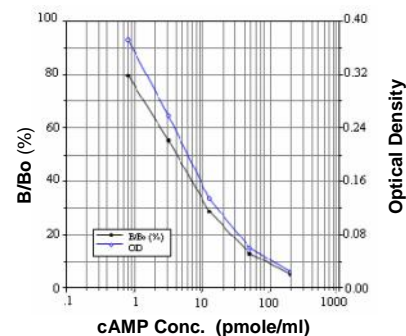
The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Non-Acetylated Version			Acetylated Version		
	Average Net OD	Percent Bound	cAMP (pmol/ml)	Average Net OD	Percent Bound	cAMP (pmol/ml)
Blank OD	(0.081)			(0.083)		
TA	0.486			0.437		
NSB	-0.001	-0.18%		0.003	0.93%	
Bo	0.471	100%	0	0.331	100%	0
S1	0.024	5.1%	200	0.017	5.2%	20
S2	0.062	13.1%	50	0.068	20.5%	5
S3	0.145	30.9%	12.5	0.127	38.5%	1.25
S4	0.272	57.9%	3.125	0.229	69.1%	0.312
S5	0.388	82.5%	0.781	0.301	90.9%	0.078
Unknown 1	0.125	26.6%	16.21	0.125	37.8%	1.45
Unknown 2	0.291	61.8%	2.57	0.276	83.5%	0.13

XIII. Typical Standard Curves and Quality Control Parameters

Typical standard curves are shown below. These curves must not be used to calculate cAMP concentrations; each user must run a standard curve for each assay and version used.

Non-Acetylated Version



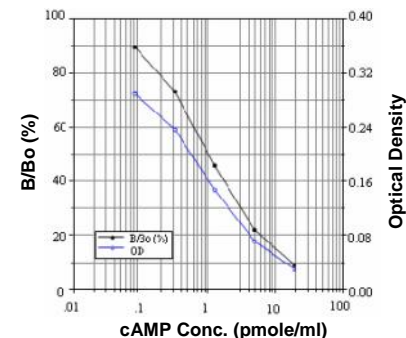
Typical Quality Control Parameters

$$\text{Total Activity Added} = 0.486 \times 10 = 4.86$$

$$\begin{aligned} \% \text{NSB} &= 0.0\% \\ \% \text{Bo/TA} &= 9.7\% \\ \text{Quality of Fit} &= 1.000 \\ &(\text{Calculated from 4 parameter logistics curve fit}) \end{aligned}$$

$$\begin{aligned} 20\% \text{ Intercept} &= 26.1 \text{ pmol/ml} \\ 50\% \text{ Intercept} &= 4.6 \text{ pmol/ml} \\ 0\% \text{ Intercept} &= 0.9 \text{ pmol/ml} \end{aligned}$$

Acetylated Version



Typical Quality Control Parameters

$$\text{Total Activity Added} = 0.437 \times 10 = 4.37$$

$$\begin{aligned} \% \text{NSB} &= 0.1\% \\ \% \text{Bo/TA} &= 7.6\% \\ \text{Quality of Fit} &= 0.998 \\ &(\text{Calculated from 4 parameter logistics curve fit}) \end{aligned}$$

$$\begin{aligned} 20\% \text{ Intercept} &= 4.5 \text{ pmol/ml} \\ 50\% \text{ Intercept} &= 0.8 \text{ pmol/ml} \\ 80\% \text{ Intercept} &= 0.2 \text{ pmol/ml} \end{aligned}$$

XIV. Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols¹⁵.

Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run with the Bo, and comparing to the average optical density for sixteen (16) wells run with Standard #5. The detection limit was determined as the concentration of cAMP measured at two (2) standard deviations from the zero along the standard curve.

Non-Acetylated Version

Mean OD for Bo = 0.340 ± 0.012 (3.4%)
 Mean OD for Standard #5 = 0.293 ± 0.007 (2.5%)
 Delta Optical Density (0–0.78 pmol/ml) = 0.340 – 0.293 = 0.047
 2 SD's of the Zero Standard = 0.024

$$\text{Sensitivity} = \frac{0.024}{0.048} \times 0.78 \text{ pmol/ml} = \mathbf{0.39 \text{ pmol/ml}}$$

Acetylated Version

Mean OD for Bo = 0.313 ± 0.004 (1.24%)
 Mean OD for Standard #5 = 0.295 ± 0.009 (2.9%)
 Delta Optical Density (0–0.078 pmol/ml) = 0.313 – 0.295 = 0.017
 2 SD's of the Zero Standard = 0.008

$$\text{Sensitivity} = \frac{0.008}{0.017} \times 0.078 \text{ pmol/ml} = \mathbf{0.037 \text{ pmol/ml}}$$

Linearity

Non-Acetylated Version

A sample containing 15.44 pmol/ml cAMP was serially diluted 4 times 1:2 in the 0.1M HCl supplied in the kit and measured in the assay. The data was plotted graphically as actual cAMP concentration versus measured cAMP concentration. The line obtained had a slope of 0.98 with a correlation coefficient of 0.988.

Acetylated Version

A sample containing 3.41 pmol/ml cAMP was serially diluted 4 times 1:2 in the 0.1M HCl supplied in the kit and measured in the Acetylated version of the assay. The data was plotted graphically as actual cAMP concentration versus measured cAMP concentration. The line obtained had a slope of 1.226 with a correlation coefficient of 0.999.

Precision

Intra-assay precision was determined by taking samples containing differing concentrations of cAMP and running these samples multiple times (n = 24) in the same assay. Inter-assay precision was determined by measuring samples with differing concentrations of cAMP in multiple assays (n = 8). The precision

numbers listed below represent the percent coefficient of variation for the concentrations of cAMP determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	Non-Acetylated Version			Acetylated Version		
	cAMP (pmol/ml)	Intra-assay (% CV)	Inter-assay (% CV)	cAMP (pmol/ml)	Intra-assay (% CV)	Inter-assay (% CV)
Low	1.24	8.9		0.679	4.6	
Medium	6.31	4.3		3.58	8.4	
High	35.29	8.3				
Low	1.18		13.1	1.29		13.6
Medium	5.52		4.2	5.62		7.8
High	30.36		11.6			

Cross Reactivities

The cross reactivities for a number of related compounds were determined by using Trevigen's HT Cyclic AMP Kit Cat # 2500-096-K, which uses the same antibody and conjugate as this kit. Potential cross reactants were dissolved in the kit Assay Buffer at concentrations from 2,000 to 2 pmol/ml. These samples were then measured in the cAMP assay, and the measured cAMP concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
cAMP	100%
AMP	0.33%
ATP	0.12%
cGMP	< 0.001%
GMP	< 0.001%
GTP	< 0.001%
cUMP	< 0.001%
CTP	< 0.001%

XV. Sample Dilution Recommendations

Please refer to pages 4 and 5 for Sample Handling recommendations and Reagent preparation. cAMP concentrations were measured in tissue culture media. cAMP was spiked into the undiluted sample which was diluted with the kit 0.1M HCl and then assayed in the kit. The following result was obtained:

Sample	Non-Acetylated Version		Acetylated Version	
	% Recovery	Recommended Dilution*	% Recovery	Recommended Dilution*
Tissue Culture Media**	85	1:5–1:20		**

*See Sample Preparation instructions on page 4 for details.

**However RPMI-1640 contains ~350 pmol/ml cAMP.

XVI. Assay Layout

Dilution Tables for Making Standards 1–5:

Non-Acetylated Version:				Acetylated Version:			
Std.	Assay Buffer 2 Vol. (µL)	Vol. Added (µL)	cAMP Conc. (pmol/mL)	Std.	Assay Buffer 2 Vol. (µL)	Vol. Added (µL)	cAMP Conc. (pmol/mL)
1	900	100, Stock	200	1	990	10, Stock	20
2	750	250, Std. 1	50	2	750	250, Std. 1	5
3	750	250, Std. 2	12.5	3	750	250, Std. 2	1.25
4	750	250, Std. 3	3.12	4	750	250, Std. 3	0.312
5	750	250, Std. 4	0.78	5	750	250, Std. 4	0.078

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
Assay Buffer 2	—	—	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	=====	=====	=====	=====	=====	50 µL
Asp. & Wash 3 × 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

cAMP Plate Layout:

A12	B12	C12	D12	E12	F12	G12	H12
A11	B11	C11	D11	E11	F11	G11	H11
A10	B10	C10	D10	E10	F10	G10	H10
A9	B9	C9	D9	E9	F9	G9	H
A8	B8	C8	D8	E8	F8	G8	H8
A7	B7	C7	D7	E7	F7	G7	H7
A6	B6	C6	D6	E6	F6	G6	H6
A5	B5	C5	D5	E5	F5	G5	H5
A4	B4	C4	D4	E4	F4	G4	H4
A3 Std 5	B3 Std 5	C3	D3	E3	F3	G3	H3
A2 Std 1	B2 Std 1	C2 Std 2	D2 Std 2	E2 Std 3	F2 Std 3	G2 Std 4	H2 Std 4
A1 Blank	B1 Blank	C1 TA	D1 TA	E1 NSB	F1 NSB	G1 B0	H1 B0

Kit Lot No. _____
 1st Incub.: Start Time _____ End Time _____ Temp. _____
 2nd Incub.: Start Time _____ End Time _____ Temp. _____
 Exp. Date _____ Temp. _____
 Date _____ Tech. _____
 Notes: _____

For a full-sized version of this chart, please visit www.trevigen.com.

XVII. References

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques, 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
3. E.W. Sutherland, G.A. Robison, and R.W. Butcher, *Circulation*, (1968) **37**: 279.
4. T.W. Rall, et al., *J. Biol. Chem.*, (1957) **224**: 463.
5. T.W. Cook, et al., *J. Am. Chem. Soc.*, (1957), **79**: 3607.
6. E.W. Sutherland, and T.W. Rall, *J. Am. Chem. Soc.*, (1957) **79**: 3608.
7. D. Lipkin, et al., *J. Am. Chem. Soc.*, (1959) **81**: 6198.
8. D. Chabardes, et al., *J. Clin. Invest.*, (1980) **65**: 439.
9. V. Grill, and E. Cerasi, *J. Biol. Chem.*, (1974) **249**: 41961.
10. R.C. Haynes, *J. Biol. Chem.*, (1958), **233**: 1220.
11. A. Szentivanyi, *J. Allergy*, (1968) **42**: 203.
12. P. Hamet, et al, *Adv. Cycl. Nucl. Res.*, (1983) **15**: 11.
13. M. Plaut, et al, *Adv. Cycl. Nucl. Res.*, (1983) **12**: 161.
14. J.H. Exton, *Adv. Cycl. Nucl. Res.*, (1983) **12**: 319.
15. NCCLS Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

XVIII. Related Products Available from Trevigen

2500-096-K HT Cyclic AMP Enzyme Immunoassay Kit 96 wells

Oxidative Damage Kits

4370-096-K	8-oxo-dG ELISA Kit	96 wells
7500-100-K	SOD Assay Kit	100 tests
7501-500-K	HT SOD Assay Kit	480 tests
7510-100-K	Glutathione Reductase Assay Kit	100 tests
7513-500-K	HT Glutathione Reductase Assay Kit	480 tests
7511-100-K	HT Glutathione Assay Kit	384 tests
7512-100-K	HT Glutathione Peroxidase Assay Kit	480 tests

www.interchim.com



211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com