TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

HT γ-H2AX Pharmacodynamic Assay

96 Tests

Cat# 4418-096-K

High throughput ELISA to quantify γ-H2AX in human cultured cells, peripheral blood mononuclear cells, and tissue biopsies

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Table of Contents

I. Introduction

Histone H2AX is a 14 kDa ubiquitous member of the H2A histone family that contains an evolutionarily conserved SQ motif at the C-terminus in eukaryotes. Serine 139 within this motif becomes rapidly phosphorylated by ATM and ATR kinases to yield a form known as γ-H2AX in response to double-strand DNA damage and apoptosis (1). During the past year investigators have confirmed the value of γ-H2AX as an important Pharmacodynamic (PD) marker (2) and genotoxicity endpoint (3). There are over 21 anticancer drugs that are known to result in γ-H2AX formation. As a result, γ-H2AX is an ideal PD surrogate marker to measure molecular responses to a large number of drugs (4,5,6). While many of these drugs have already garnered regulatory approval, and are currently being used to manage various types of cancers, they are the subject of ongoing clinical studies to evaluate their efficacy when used alone or in combination with molecularly targeted drugs.

While methods such as western blots and immunohistochemistry are widely used but difficult to validate to regulatory standards, the ELISA method is the most quantifiable and easiest to validate. To address this need Trevigen's quantitative pharmacodynamic HT γ-H2AX assay measures γ-H2AX levels in cellular extracts and phosphorylation of H2AX in response to therapeutic intervention. This assay documents differences of γ-H2AX levels in human PBMC, cultured cells, tissue biopsies, and will be useful in future clinical trials providing one of many needed tools to enable hypothesis-driven preclinical drug design strategies.

Immobilized γ-H2AX antibody in the wells of a 96-well plate captures γ-H2AX from sample lysate. Incubation with a H2AX detecting antibody, followed by addition of a Goat anti-mouse HRP conjugate and a chemiluminescent HRP substrate yields relative light units (RLU) that directly correlates with the amount of γ-H2AX in the sample. This assay is ideal for quantification of γ-H2AX in human peripheral blood mononuclear cells, tissue biopsies, and cultured cells. Important features of the assay include: 1) chemiluminescent, non-radioactive format; 2) high throughput 96 test format with pre-coated capture antibody; 3) dynamic range from 10 pM to 800 pM; and, 4) sensitivity with 5 pM of γ-H2AX.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of the chemicals and reagents contained in the HT γ-H2AX Pharmacodynamic Assay may not yet have been fully investigated. Therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using any of these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products. MSDS are available on request.
3. The components in each kit lot number have been quality assured and validated in this specific combination only; please do not mix them with components from other kit lot numbers.

III. Materials Supplied

Catalog Number	Component	Amount Provided	Storage
4418-096-P	96-stripwell pre-coated plate with 3 film sealers	1 plate	4°C
4418-096-01	γ-H2AX Standard, 1 µM	20 µl	-20°C
4418-096-02	Assay Buffer	50 ml	4°C
4418-096-03	H2AX IgM Detecting Antibody	30 µl	-20°C
4418-096-04	Goat anti-Mouse IgM HRP Conjugate	15 µl	-20°C
4418-096-05	Cell Lysis Reagent	40 ml	4°C
4418-096-06	Jurkat Cell Lysate Control	60 µl	-20°C
4418-096-07	25X Wash Buffer	40 ml	RT
4675-096-01	PeroxyGlow™ A	6 ml	4°C
4675-096-02	PeroxyGlow™ B	6 ml	4°C

IV. Materials/Equipment Required But Not Supplied

Reagents/Disposables:

1. Biological specimens to be tested
2. Distilled water
3. 200 mM Phenylmethyl Sulfonyl Fluoride (PMSF) in ethanol
4. Protease Inhibitor Cocktail (Thermo Fisher Scientific, Cat# 78429, optional)
5. 1 - 200 µl and 100-1000 µl pipette tips
6. PBS and/or Plasma Lyte A (Baxter HealthCare, Corp. Cat# 2B2544X)

Equipment:

1. Micropipettes and tips
2. Multichannel pipettor 10 µl - 100 µl
3. Wash bottle or microstrip wells plate washer (optional)
4. BD Vacutainer® CPT™ (Na HEPARIN) (Ref 362753)
5. 96-well chemiluminescent plate reader or luminometer
6. Refrigerated centrifuge with swinging bucket rotor
7. Microcentrifuge
8. 15 ml and 50 ml screw cap centrifuge tubes
9. 0.5 ml and 1.5 ml microtubes
10. 25 ml solution reservoirs
11. Incubator set at 25 °C
12. Refrigerator
13. -20 °C freezer

V. Reagent Preparation

1. 1X Wash Buffer (TBST: TBS + 0.1% Tween 20)

Prepare 1 liter of 1X Wash Buffer with provided 25X Wash Buffer (Cat# 4418-096-07) using deionized water in wash bottle for washing strip wells.

2. 96-stripwell pre-coated plate

γ-H2AX antibody pre-coated white strip wells (Cat# 4418-096-P) are provided for convenience with 3 film sealers.

3. γ-H2AX Standard

The kit contains 20 µl of γ-H2AX Standard (Cat# 4418-096-01) at a concentration of 1 µM. Centrifuge before opening vial and aliquot to avoid repeated freeze/thaw cycles.

The standard curve requires 50 µl/well of each dilution and each is recommended in triplicate. The recommended final concentrations are 800, 400, 200, 100, 50, 20, and 10 pM.

A. Prepare 40 nM γ-H2AX by diluting 1µM γ-H2AX Standard (Cat# 4418-096-01) in Assay Buffer. Example: Add 3 µL of 1 µM Standard to 72 µL Assay Buffer and mix well.

B. Prepare a series of diluted γ-H2AX standards using 40 nM of γ-H2AX according to Table 1.

Note: Diluted γ-H2AX (including 40 nM of γ-H2AX) standards should be used immediately and any remainder discarded.

Table 1: γ-H2AX Standard Curve

Tube #	γ-H2AX Dilutions	Assay Buffer	Final γ-H2AX
1	8 µl 40 nM γ-H2AX	392 µl	800 pM
2	200 µl tube #1	200 µl	400 pM
3	200 µl tube #2	200 µl	200 pM
4	200 µl tube #3	200 µl	100 pM
5	200 µl tube #4	200 µl	50 pM
6	160 µl tube #5	240 µl	20 pM
7	200 µl tube #6	200 µl	10 pM

4. H2AX IgM Detecting Antibody

Gently dilute the H2AX Detecting Antibody (cat# 4418-096-03) 250-fold with Assay Buffer by pipetting (Note: avoid vortexing or vigorous mixing). A total of 50 µl/well of diluted H2AX IgM Detecting Antibody is required in the assay. For a plate, dilute 24 µl of H2AX IgM Detecting Antibody into 6 ml of Assay Buffer then add 50 µl/well with a multichannel pipettor.

5. Goat anti-Mouse IgM HRP Conjugate

Gently dilute the Goat anti-Mouse IgM HRP conjugate (cat# 4418-096-05) 1000-fold with Assay Buffer. A total of 50 µl/well of diluted Goat anti-mouse IgM HRP Conjugate is required in the assay. For a plate dilute 6 µl of Goat anti-Mouse IgM HRP Conjugate into 6 ml of Assay Buffer then add 50 µl/well with a multichannel pipettor.

6. PeroxyGlow™ A and B Chemiluminescent Substrates

Equilibrate to room temperature PeroxyGlow™ A and B before use. Immediately before addition (Section VII step 10), mix equal volumes of PeroxyGlow™ A and B together. A total of 100 µl is required per well. PeroxyGlow™ A and B are Horseradish Peroxidase (HRP) substrates generating light that can be quantified in a suitable chemiluminescent plate

reader. For a plate mix 6 ml of PeroxyGlow™ A with 6 ml of PeroxyGlow™ B then add 100 µl/well with a multichannel pipettor.

7. Cell Lysis Buffer

Just prior to use, prepare 1 ml of the following Cell Lysis Buffer and place on ice:

Cell Lysis Reagent* (cat# 4418-096-06)	995 µl
200 mM PMSF** (in ethanol)	5 µl

* Cell Lysis Reagent contains phosphatase inhibitors.
** The addition of Protease Inhibitor Cocktail (Thermo Scientific) is optional.

8. Jurkat Cell Lysate Control

Upon dilution in Assay Buffer, Jurkat Cell Lysate Control contains 100 pM - 300 pM γ-H2AX when diluted 1:10. Store lysate at -20 °C in working aliquots and avoid repeated freeze/thaw cycles. Keep on ice after thawing.

VI. Preparation of Cell Extracts

i. Suspension cells*:*

1. Grow 1-5 x 10^6 suspension cells in complete medium in a suitable tissue culture plate or flask.
2. Transfer cells to prechilled 50 ml screw cap tube. Centrifuge at 200 x g for 3 min at 4°C and discard supernatant.
3. Suspend cells in 10 ml of ice-cold 1X PBS and perform cell count.
4. Centrifuge at 200 x g for 3 min at 4°C and discard supernatant.
5. Suspend cell pellet in 1 ml of ice-cold 1X PBS and transfer to 1.5 ml microtube. Centrifuge at 10,000 x g or top speed for 10 sec at 4°C. Discard supernatant. **Note:** If not used immediately, flash-freeze cell pellet in liquid nitrogen and store at -80 °C.
6. Suspend cell pellet at 5 x 10^6 cells/ml in Cell Lysis Buffer (Section V item 7). For example, add 1 ml cold Cell Lysis Buffer to 5 x 10^6 cell pellet and pipette up and down to suspend cells.
7. Incubate on ice for 20 minutes and vortex periodically.
8. Centrifuge at 10,000 x g for 10 minutes at 4 °C. Carefully transfer supernatant to new 1.5 ml tube.
9. Measure the protein concentration of extracts by BCA protein assay.
10. Assay extract immediately or aliquot and store at -20 °C.

ii. Adherent cells*:*

1. Grow 1-5 x 10^6 adherent cells in complete medium in a suitable tissue culture plate until 70-80% confluent.
2. Remove media and gently wash cells with 5 ml of 37°C PBS. Repeat PBS wash one more time. Detach the cells with trypsinization according to standard procedure.
3. Suspend cells in 10 ml of ice-cold 1X PBS and perform cell count.
4. Centrifuge at 200 x g for 3 min at 4°C and discard supernatant.
5. Suspend cell pellet in 1 ml of ice-cold 1X PBS and transfer to 1.5 ml microtube. Centrifuge at 10,000 x g or top speed for 10 sec at 4°C. Discard

supernatant. **Note:** If not used immediately, flash-freeze cell pellet in liquid nitrogen and store at -80 °C.

6. Suspend cell pellet at 5 x 10^6 cells/ml in Cell Lysis Buffer (Section V item 7). For example, add 1 ml cold Cell Lysis Buffer to 5 x 10^6 cell pellet and pipette up and down to suspend cells.
7. Incubate on ice for 20 minutes and vortex periodically.
8. Centrifuge at 10,000 x g for 10 minutes at 4 °C. Carefully transfer supernatant to new 1.5 ml tube.
9. Measure the protein concentration of the extracts by BCA protein assay.
10. Assay immediately or aliquot and store at -20 °C.

iii. Peripheral Blood Mononuclear Cells (PBMC):

1. Withdraw 8 ml of blood into BD Vacutainer® CPT™ tube containing Sodium Heparin as the anticoagulant. Mix by gently inverting 8-10 times.
2. Centrifuge at room temperature (18-25 °C) in a horizontal rotor (swinging bucket) with proper adaptor for 25 minutes at 1500 x g.
3. After centrifugation, carefully transfer PBMC layer (whitish layer under the plasma layer) into 50 ml conical tube.
4. Add Plasma Lyte A to 40 ml final volume and mix by inverting 5-8 times.
5. Centrifuge at 300 x g for 10 minutes at 25 °C and discard supernatant.
6. Suspend cell pellet in cold Plasma Lyte A to 10 ml final volume and perform cell count.
7. Centrifuge at 300 x g for 10 minutes at 25 °C and discard supernatant.
8. Suspend cell pellet in 1 ml Plasma Lyte A, transfer to 1.5 ml microtube and place on ice.
9. Centrifuge at 10,000 x g for 10 sec at 4°C and discard supernatant.
   Note: If not used immediately, flash-freeze cell pellet in liquid nitrogen and store at -80 °C.
10. Suspend cell pellet at 5x10^6 cells/ml in Cell Lysis Buffer (Section V item 7). For example, add 1 ml Cell Lysis Buffer to 5 x 10^6 cell pellet and pipette up and down to suspend cells.
11. Incubate on ice for 20 minutes and vortex periodically.
12. Centrifuge at 10,000 x g for 10 minutes at 4 °C. Carefully transfer supernatant to new 1.5 ml tube.
13. Measure protein concentration of extract by BCA protein assay.
14. Assay immediately or aliquot and store at -20 °C.

iv. Tissues Biopsies

1. Biopsy of tissue samples are collected according to standard procedure and specimen sizes are typically range from 5-20 mm in length, 3 to 12 mg in mass. If not used immediately, snap-freeze in liquid nitrogen and store at -80 °C until assayed.
2. Add 1 ml cold Lysis Buffer (section V item 7) to frozen tissue and mince completely with fine-point scissors. Vortex to mix and place on ice. Pipette up and down to suspend cells.
3. Incubate on ice for 20 minutes and vortex periodically.
4. If necessary, disrupt the extracts by sonication on ice three times for 10 seconds each cycle. Vortex and place on ice for 15 min.

5. Centrifuge at 10,000 x g for 10 minutes at 4 °C. Carefully transfer supernatant to new 1.5 ml tube.
6. Measure protein concentration of extract by BCA protein assay. Assay immediately or aliquot and store at -20 °C.

v. High Throughput Screening

1. For high throughput screening purpose, cells can also be seeded into suitable 96-well plate at 10,000-50,000 cells/well. Culture cells per manufacturer's recommendation; adherent cells should be cultured to no more than 80% confluence.
2. Centrifuge at 200 x g for 3 min at 4°C with a 96-well plate adapter and discard supernatant. Suspend cells in ice-cold 1X PBS. Centrifuge at 200 x g for 3 min at 4°C and discard supernatant. Repeat PBS wash one more time. For adherent cells directly remove media and gently wash cells with ice-cold 1X PBS. Gently remove PBS and repeat PBS wash one more time.
3. Centrifuge at 200 x g for 3 min at 4°C and discard supernatant.
4. Immediately add 10-20 µl Cell Lysis Buffer (Section V item 7) to each well. Incubate on ice or 4 °C for 20 minutes and carefully pipetting the lysate periodically to avoid air bubbles.
5. Transfer the lysate from the wells and assay immediately according to VII Assay Protocol or store lysate at -20 °C for later use.

VII. Assay Protocol

See Section XI Appendix for Sample Plate Layout.

1. Bring Assay Buffer, PeroxyGlow™ A and B to room temperature. Remove 96-stripwell pre-coated plate from foil pouch and bring to room temperature.

   Note: If fewer wells are needed, place excess stripwells in foil pouch and store at 4 °C with desiccant. (Data performance will be compromised if desiccant color changes from blue to pink).

2. Prepare dilutions of γ-H2AX Standard (Section V item 3) for standard curve and Jurkat Cell Lysate for positive control (Section V item 8).

3. Prepare dilutions of Sample Extracts (Section VI) to fall within standard curve using 25°C Assay Buffer (cat# 4418-096-02).

   NOTES:

   i. Recommend diluting cell sample extracts in the starting range of 20,000 cells/well to 50,000 cells/well. Cell samples are added in 50 µl volume. γ-H2AX levels in PBMC, suspension, and adherent sample extracts are reported as pM γ-H2AX per 10^7cells/ml extract.

   ii. Recommend diluting tissue biopsy extracts in the starting range of 0.5 µg/well to 2 µg/well. Tissue biopsy samples are added in 50 µl volume. γ-H2AX levels in tissue cell extracts are reported as pM γ-H2AX per mg/ml of protein extract.

iii. If samples generate values greater than the 800 pM standard, assay at a higher sample dilution. If samples generate values lower than 10 pM standard, assay at a lower sample dilution.

4. Add 50 µl/well of γ-H2AX standards (Section V item 3), diluted test samples, diluted Jurkat Cell Lysate Control (Section V item 8), and Assay Buffer (background control) to appropriate wells in triplicate. Apply film sealer and incubate at 25 °C for 2 hours. Gently dilute H2AX IgM detecting antibody 1:250 fold in Assay Buffer by pipetting (avoid vortexing or vigorous mixing) (Section V item 4) **and incubate on ice or 4 °C half hour before use.**

5. Gently remove plate sealer and rinse wells 4 times with 1X Wash Buffer (300 µl well, Section V item 1). Ensure that all liquid is removed by tapping strip wells onto paper towels.

6. Add 50 µl per well of diluted H2AX IgM Detecting Antibody. Apply film sealer and incubate at 25 °C for 1 hour.

7. Gently remove the plate sealer and rinse wells 4 times with 1X Wash Buffer (300 µl/well). Ensure that all the liquid is removed by tapping strip wells onto paper towels.

8. Add 50 µl per well of diluted Goat anti-Mouse IgM HRP Conjugate (Section V item 5). Apply film sealer and incubate at 25°C for 1 hour. Place PeroxyGlow™ A and B reagents at 25 °C to pre-warm.

9. Gently remove the plate sealer and rinse wells 4 times with 1X Wash Buffer (300 µl/well). Ensure that all the liquid is removed by tapping strip wells onto paper towels.

10. Just before use, mix equal volumes of PeroxyGlow™ A and B together and add 100 µl per well. Immediately take chemiluminescent readings.

VIII. Data Interpretation

Determination of γ-H2AX concentrations on the Web

Use Calculations Worksheet provided on Trevigen website: http://www.trevigen.com/gammaH2AX-ELISA.php. (MS Excel is required).

Alternative Method to Determine γ-H2AX concentrations

1. Calculate the average LU measurement for each γ-H2AX Standard, sample and blank.
2. Subtract the blank average LU from γ-H2AX Standard and sample averages to determine the relative RLU.
3. Plot the γ-H2AX Standard concentrations (pM) on the X-axis versus their relative RLU on the Y-axis.
4. The standard curve is a 2nd order polynomial function represented by the equation: $y = a + bx + cx^2$, where y is the relative RLU, x is γ-H2AX standard γ-H2AX concentrations using the polynomial equation or interpolate from the standard curve.

5. To determine final γ-H2AX sample concentrations, multiply by the sample dilution. For example, if the sample was diluted 1:10, the value generated from the polynomial equation or the standard curve must be multiplied by 10 to determine the final sample γ-H2AX concentration.

Example data

The following figures are HT γ-H2AX Pharmacodynamic Assay examples. The data below is for reference only and should not be used to interpret actual results.

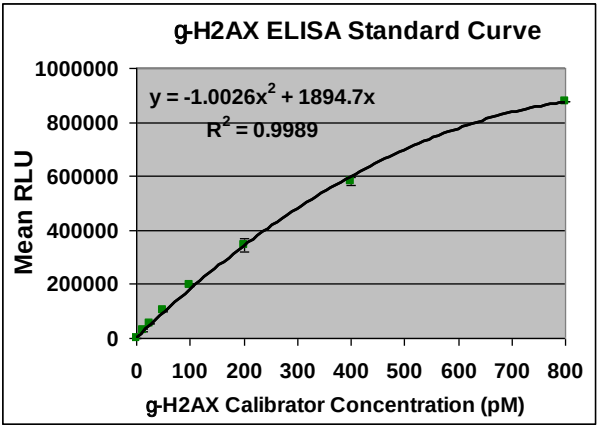

Figure 1. Typical Standard Curve. Standards were prepared in Section V and assayed according to the protocol in Section VII.

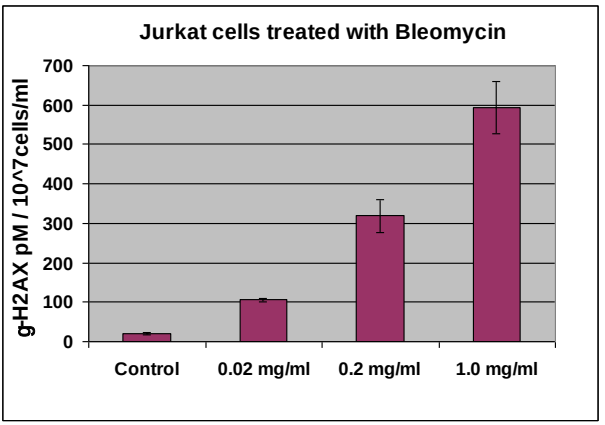

Figure 2. γ-H2AX values from Jurkat Cells treated with Bleomycin. Jurkat cells treated at $5X10^6$ cells/ml with 0.02 mg/ml, 0.2 mg/ml and 1.0 mg/ml of Bleomycin respectively and lysed according to section VI.

IX. Performance Characteristics

Intra-Assay Precision: The intra-assay precision (within-run precision): three samples of known concentration were tested nine times in a single run. The overall intra-assay coefficient of variation was calculated to be <10%.

Inter-Assay Precision: The inter-assay precision (between-run precision): three samples of known concentration were tested nine times in three separate runs. The overall inter-assay coefficient of variation was calculated to be <10%.

Sensitivity of the ELISA: The LLD (low limit of detection) of γ-H2AX was calculated to be 5 pM.

Spike and Recovery: Test samples were spiked with three different levels of γ-H2AX and analyzed for recovery before and after spiking. The calculated overall mean of assay accuracy is between 100% ± 10%.

Dilution Linearity: Test samples were serially diluted in the Assay Buffer and subsequently measured by the assay. Dilution recovery is assessed by comparing observed vs. expected values based on undiluted samples. The calculated overall mean of dilution recovery is between 100% ± 10%.

Specificity: The assay cross-reactivity test is performed by phosphorylated peptide and non-phosphorylated H2AX peptide: the assay only specifically detects phosphorylated peptide and has no cross reactivity with non-phosporylated H2AX peptide.

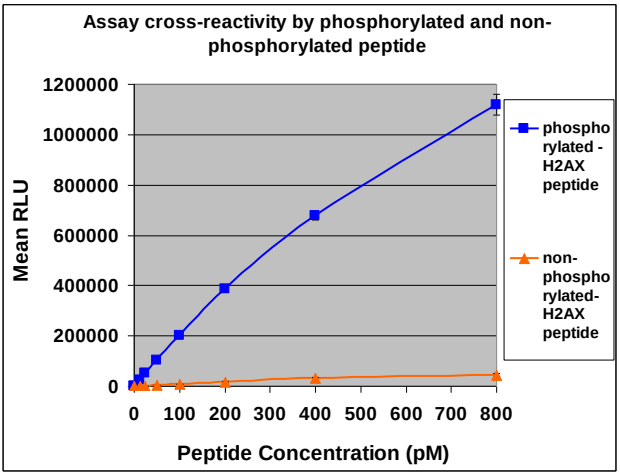

Figure 3. γ-H2AX assay cross-reactivity. Assay specificity was tested by using phosphorylated peptide and non-phosphorylated H2AX peptide: the assay shows no cross reactivity with non-phosporylated H2AX peptide.

X. References

1. E.P. Rogakou, D.R. Pilch, A.H. Orr, V.S. Ivanova, and W.M. Bonner. DNA Double-stranded Brekas Induce Histone H2AX phosphorylation on Serine 139. *The Journal of Biological Chemistry* (1998) Vol 273, No.10, 5858–5868.
2. Wu, J. Clingen, PH., Spanswick, VJ., Mellinas-Gomez, M., Meyer T., Puzanov, I., Jodrell, D., Hochhauser, D. and Hartley JA (2013) *Clinical Cancer Research*, 19, 721-730.
3. Watters GP, Smart DJ, Harvey JS, Austin CA (2009) H2AX phosphorylation as a genotoxicity endpoint. *Mutat Res*, 679, 50-58.
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5. Kinders RJ, Hollingshead M, Lawrence S, Ji J, Tabb B, Bonner WM, Pommier Y, Rubinstein L, Evrard YA, Parchment RE, Tomaszewski J, Doroshow JH (2010) *Clin Cancer Res*. 16(22):5447-57.
6. Redon CE, Nakamura AJ, Martin OA, Parekh PR, Weyemi US, Bonner WM. (2011*) Aging (Albany NY).* 3(2):168-74.

XI. Related Products Available From Trevigen

Kits:

Catalog #	Description	Size
4250-050-K	CometAssay Kit	50 samples
4253-096-K	CometAssay Kit 96 wells	96 samples
4520-096-K	HT PARP in vivo Pharmacodynamic Assay II	96 samples
4380-096-K	8 oxodG ELISA kit II	96 tests
4380-192-K	8 oxodG ELISA Kit II	2X96 tests
4700-096-K	Tankyrase 1 Colorimetric Activity Assay	96 tests
4701-096-K	Tankyrase 1 Chemiluminescent Activity Assay	96 tests
4360-096-K	BPDE ELISA Kit	96 tests
4676-096-K	Universal Chemiluminescent PARP Assay Kit/w Histone Coated Strip Wells	96 samples
4677-096-K	Universal Colorimetric PARP Assay Kit/w Histone Coated Strip Wells	96 samples
4684-096-K	HT Colorimetric PARP Apoptosis Assay	96 samples
4685-096-K	HT Chemiluminescent PARP Apoptosis Assay	96 samples
4690-096-K	HT F Homogeneous PARP Inhibition Assay Kit	96 tests
4691-096-K	HT Homogeneous PARG Inhibition Assay	96 tests
4682-096-K	HT Chemiluminescent PARG Assay Kit	96 tests
4683-096-K	HT Colorimetric PARG Assay Kit	96 tests

Antibodies:

Catalog #	Description	Size
4118-APC-100	Anti-Phosphorylated H2AX Affinity Purified Polyclonal	100 µl
4118-APC-020	Anti-Phosphorylated H2AX Affinity Purified Polyclonal	20 µl
4335-AMC-50	PAR Monoclonal Antibody Affinity Purified	50 µl
4335-MC-100	Anti-PAR Monoclonal Antibody	100 µl
4336-APC-50	PAR Polyclonal Antibody Affinity Purified	50 µl
4336-BPC-100	Anti-PAR Polyclonal Antibody (rabbit)	50 µl
4338-MC-50	Anti-PARP Monoclonal Antibody (clone C2-10)	50 µg
2305-PC-100	Anti-Cleaved Caspase 3 Polyclonal	100 µl

XII. Appendix

Table 2: Sample Plate Layout

	1-3	3-6	7-9	10-12
A	γ-H2AX 800	Sample 1	Sample 8	Sample 15
B	γ-H2AX 400	Sample 2	Sample 9	Sample 16
C	γ-H2AX 200	Sample 3	Sample 10	Sample 17
D	γ-H2AX 100	Sample 4	Sample 11	Sample 18
E	γ-H2AX 50	Sample 5	Sample 12	Sample 19
F	γ-H2AX 20	Sample 6	Sample 13	Sample 20
G	γ-H2AX 10	Sample 7	Sample 14	Jurkat Control
H	γ-H2AX 0	Assay Buffer	Assay Buffer	Assay Buffer

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

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