# Europium Tetracycline (EuTc)

## EuTc-Hydrogen Peroxide Assay

(version A3)

Catalog No. 15003

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## Introduction

Hydrogen peroxide ($H_2O_2$) is a reactive metabolic by-product that is a key regulator in a number of oxidative stress-related states. Functioning through NFκB and other factors, hydroperoxide-mediated pathways have been linked to asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. Due to its implication in these many disease states, there is much interest in sensitive assays to monitor hydrogen peroxide in biochemical, clinical, as well as in environmental samples. In addition, there is much research on the enzymes that produce and eliminate hydrogen peroxide, and on their inhibitors.

Active Motif's EuTc-Hydrogen Peroxide Assay is based on a novel fluorescent reagent that is used to detect free or enzymatically produced $H_2O_2$. Europium Tetracycline (EuTc) detects hydrogen peroxide in a fast and easy method, and without the need for additional enhancer solutions. This makes EuTc a sensitive reagent to monitor catalases, peroxidases or glucose-oxidase activities through *in vitro* enzymatic assays, or to study substances that inhibit or activate these enzymes. The main advantages of using EuTc for luminescent peroxide detection are:

- Fast, 10-minute incubation time
- Applicable to turbid samples
- Functions at neutral pH
- No need for enzymatic amplification
- Large Stoke's shift decreases interference of background fluorescence
- Long decay time enables time-resolved, or time-gated, measurement

| product | format | catalog no. |
|---|---|---|
| EuTc | 1000 rxns* | 15003 |

*Sufficient EuTc is provided for 1000 rxns performed in 96-well plates, or 100 rxns in cuvettes.

**The EuTc-$H_2O_2$ Assay is for research use only. Not for use in diagnostic procedures.**

## Assay Principles and Performance

Europium tetracycline (EuTc) alone is weakly fluorescent. But, it combines rapidly with free $H_2O_2$ to form a EuTc-$H_2O_2$ complex that is 15-fold brighter than EuTc alone (Figure 1) Thus, the basic principle of EuTc assays is based upon the following equilibrium:

$$\underset{\text{(weakly fluorescent)}}{\text{EuTc} + H_2O_2} \longleftrightarrow \underset{\text{(strongly fluorescent)}}{\text{EuTc-}H_2O_2}$$

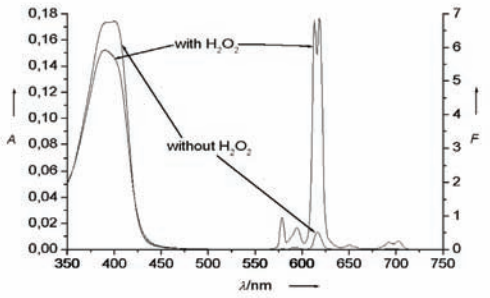


**Figure 1: Absorption and emission spectra of EuTc before and after addition of hydrogen peroxide.**

In addition to measuring $H_2O_2$ levels via the increase in fluorescent intensity, EuTc makes it possible to measure the change in decay time (lifetime) of the EuTc-$H_2O_2$ complex (Table 1). The use of lifetime-based measurement has significant advantages when measuring hydrogen peroxide levels in biological fluids as such samples typically have high levels of auto-fluorescence that can interfere with intensity-based measurements. Because the EuTc-$H_2O_2$ signal is relatively long lived, lifetime measurement can completely eliminate any background contributed by the light source, the biological sample (typically ~0.1-10 ns) or by free EuTc.

| Dye Complex | Absorption (nm) | Emission (nm) | Time (µs) |
|---|---|---|---|
| EuTc | 406 | 617 | 30 |
| EuTc-$H_2O_2$ | 406 | 617 | 60 |

**Table 1: Lifetime measurements of free and peroxide-bound EuTc.**

**Assay time:** 30 minutes.

**Detection limit:** 0.96 µM of $H_2O_2$.

**Interferences:** Phosphate and citrate cause an increase in fluorescence intensity of EuTc and may interfere with the increase in fluorescence caused by EuTc- $H_2O_2$-complex formation. Detergents should also be avoided as they can interfere with the EuTc reagent.

## Components and Storage

EuTc is supplied as a dry powder that can be stored at room temperature. After resuspension, it should be stored at -4°C in the dark.

| Reagents | Quantity | Storage / Stability |
|---|---|---|
| EuTc reagent | 1000 rxns | Room Temp for 6 months |

**Additional materials required for both 96-well plate and cuvette assays**

- 1 ml of 30% $H_2O_2$
- Distilled water

**Additional materials required for 96-well plate assays only**

- 6 x 1 ml reaction tubes
- Reaction tubes, 2 ml minimum
- Fluorescence microplate reader, excitation of 395-405 nm / emission of 617 nm

**Additional materials required for cuvette assays only**

- 6 x 10 ml glass flasks
- Reaction tubes, 2 ml minimum
- Cuvettes
- Fluorescence reader, excitation of 395-405 nm / emission of 617 nm

## Protocols

## A. Buffer Preparation and Recommendations

**Preparation of Solution A (EuTc Working Solution)**

- Dissolve the entire amount of the supplied EuTc in 100 ml of distilled water.
- Its absorbance at 405 nm is ~0.76 per cm.
- Store the EuTc Working Solution at 4°C in the dark.
- EuTc Working Solution is stable for one month if kept in the dark.

**Preparation of $H_2O_2$ Stock Solution**

- Add 810 µl of 30% hydrogen peroxide into a final volume of 10 ml distilled water.
- $H_2O_2$ Stock Solution is stable for one month when kept at 4°C

**Preparation of Solution B (400 µM hydrogen peroxide standard)**

- Add 50 µl of $H_2O_2$ Stock Solution (prepared above) into a final volume of 100 ml distilled $H_2O$.
- Solution B should be prepared freshly for each new experiment.

## B. Cuvette Assay

**Preparation of the Calibration Graph**

1. Make 6 dilutions of Solution B in distilled water in 10 ml flasks at room temperature:

| Reagent | 1 (400 µM) | 2 (320 µM) | 3 (200 µM) | 4 (80 µM) | 5 (40 µM) | 6 (20 µM) |
|---|---|---|---|---|---|---|
| Solution B | 10 ml | 8 ml | 5 ml | 2 ml | 1 ml | 0.5 ml |
| $dH_20$ | 0 ml | 2 ml | 5 ml82 ml | 1 ml | 9 ml | 9.5 ml |
| **Total Volume** | **10 ml** | **10 ml** | **10 ml** | **10 ml** | **10 ml** | **10 ml** |

2. Add 1 ml each of the diluted solutions to a 2 ml reaction tube. Work at least in duplicates.
3. Add 1 ml of Solution A to each tube, mix by pipetting up and down, then incubate for 10 minutes at room temperature.
4. In a separate tube, mix 1 ml of distilled water with 1 ml of Solution A for use as a blank.
5. Pipette each reaction mix into a cuvette and measure the luminescence intensity against the blank. Choose an excitation wavelength of 395-405 nm and an emission wavelength of 617 nm (at 8-10 nm bandwidth).
6. Calculate the average value for the duplicate measurements, then plot the fluorescence intensity (or relative increase in fluorescence intensity) versus the concentration of $H_2O_2$.

**Determination of $H_2O_2$ Concentrations in Samples**

1. Mix 1 ml of aqueous sample containing 0.1-10 mg/ml of $H_2O_2$ with 1 ml of Solution A in a 2 ml reaction tube and incubate for 10 minutes at room temperature. As phosphate buffer can cause high background, we recommend samples in phosphate-free buffers like MOPs.
2. Pipette each solution into a cuvette and measure the luminescence as described above.
3. Use the Calibration Graph prepared above and the measured luminescence to determine the concentration of $H_2O_2$ for each sample.

## C. 96-well Microplate Assay

**Preparation of the Calibration Graph**

1. Make 6 dilutions of Solution B in distilled water in 1 ml tubes at room temperature:

| Reagent | 1 (400 µM) | 2 (320 µM) | 3 (200 µM) | 4 (80 µM) | 5 (40 µM) | 6 (20 µM) |
|---|---|---|---|---|---|---|
| Solution B | 1000 µl | 800 µl | 500 µl | 200 µl | 100 µl | 50 µl |
| $dH_20$ | 0 µl | 200 µl | 500 µl | 800 µl | 900 µl | 950 µl |
| **Total Volume** | **1 ml** | **1 ml** | **1 ml** | **1 ml** | **1 ml** | **1 ml** |

2. Pipette 100 µl each of the diluted solutions into a well of a 96-well microplate. Work at least in duplicates.
3. Add 100 µl of Solution A to each tube, mix and incubate for 10 minutes at room temperature.
4. In a separate wells, mix 100 µl of distilled water with 100 µl of Solution A for use as a blank.
5. Read the plate on a fluorescence microplate reader set to an excitation wavelength of 395-405 nm and an emission wavelength of 617 nm. If a time-resolving reader is available, set the lag time to >30 µs and integrate over 100 µs.
6. Calculate the average value for the duplicate measurements, then plot the fluorescence intensity (or relative increase in fluorescence intensity) versus the concentration of $H_2O_2$.

**Determination of $H_2O_2$ Concentrations in Samples**

1. Mix 100 µl of aqueous sample containing 0.2-10 mg/ml of $H_2O_2$ with 100 µl of Solution A in a well of a 96-well microplate and incubate for 10 minutes at room temperature. As phosphate buffer can cause high background, we recommend samples in phosphate-free buffers like MOPs.
2. Measure the luminescence as described above.
3. Use the Calibration Graph prepared above and the measured luminescence to determine the concentration of $H_2O_2$ for each sample.

# Appendix

## Section A. Troubleshooting Guide

| Problem/question | Recommendation |
|---|---|
| High background in samples | Phosphate and citrate can cause increases in the fluorescent intensity of the EuTc-$H_2O_2$ complex. Use phosphate-free buffer systems like MOPs buffer. |
| | Set the lag time of the reader above 30 µs. |
| High fluorescent signal in "negative" controls | It is important to prepare the Standard Curve in the same media as that of the sample. Use equal volumes of buffer in the sample and in the dilutions when preparing the Standard Curve. |

## Section B. Related Products

| MAX Stain™ Immunofluorescence Tools | Format | Catalog No. |
|---|---|---|
| MAXpack™ Immunostaining Media Kit (contains 1 each of 15252, 15253 & 15254) | 1 kit | 15251 |
| MAXblock™ Blocking Medium | 150 ml | 15252 |
| MAXbind™ Staining Medium | 250 ml | 15253 |
| MAXwash™ Washing Medium | 1000 ml | 15254 |

| Cell Viability Assay | Format | Catalog No. |
|---|---|---|
| ToxCount™ Cell Viability Assay | 20 x 96 rxns | 18010 |

| Transcription Factor ELISAs | Format | Catalog No. |
|---|---|---|
| Albumin Blue Fluorescent Assay Kit | 1 kit | 15002 |

| Fluorescent Cell Stains | Format | Catalog No. |
|---|---|---|
| LavaCell™ Live Cell Membrane Staining Kit | 200 µg | 15004 |
| Chromeo™ Live Cell Mitochondrial Staining Kit | 1 kit | 15005 |
| Chromeo™ Red Fluorescent Fixed Cell Staining Kit | 1 kit | 15006 |

| Protein & Extract Quantification | Format | Catalog No. |
|---|---|---|
| ProStain™ Protein Quantification Kit | 1000 rxns | 15001 |

| Capillary Electrophoresis | Format | Catalog No. |
|---|---|---|
| CE Dye 503 | 1 kit | 15101 |
| CE Dye 540 | 1 kit | 15102 |

| Fluorescent Pyrylium Dyes (Py-Dyes) | Format | Catalog No. |
|---|---|---|
| Chromeo™ P429 | 1 mg | 15108 |
| Chromeo™ P465 | 1 mg | 15105 |
| Chromeo™ P503 | 1 mg | 15106 |
| Chromeo™ P540 | 1 mg | 15107 |
| Chromeo™ P543 | 1 mg | 15109 |

| Fluorescent Dyes | Excitation / Emission | Format | Catalog No. |
|---|---|---|---|
| Chromeo™ 488 Carboxylic Acid | 488 nm / 517 nm | 1 mg | 15510 |
| Chromeo™ 488 NHS-Ester | 488 nm / 517 nm | 1 mg | 15511 |
| Chromeo™ 494 Carboxylic Acid | 494 nm / 628 nm | 1 mg | 15110 |
| Chromeo™ 494 NHS-Ester | 494 nm / 628 nm | 1 mg | 15111 |
| Chromeo™ 505 Carboxylic Acid | 505 nm / 526 nm | 1 mg | 15610 |
| Chromeo™ 505 NHS-Ester | 505 nm / 526 nm | 1 mg | 15611 |
| Chromeo™ 546 Carboxylic Acid | 545 nm / 561 nm | 1 mg | 15210 |
| Chromeo™ 546 NHS-Ester | 545 nm / 561 nm | 1 mg | 15211 |
| Chromeo™ 642 Carboxylic Acid | 642 nm / 660 nm | 1 mg | 15310 |
| Chromeo™ 642 NHS-Ester | 642 nm / 660 nm | 1 mg | 15311 |

| Antibody/Protein Labeling | Excitation / Emission | Format | Catalog No. |
|---|---|---|---|
| Chromeo™ 488 Antibody Labeling Kit | 488 nm / 517 nm | 1 kit | 15090 |
| Chromeo™ 494 Antibody Labeling Kit | 494 nm / 628 nm | 1 kit | 15091 |
| Chromeo™ 546 Antibody Labeling Kit | 545 nm / 561 nm | 1 kit | 15092 |
| Chromeo™ 642 Antibody Labeling Kit | 642 nm / 660 nm | 1 kit | 15093 |

| Fluorescent Secondary Antibodies | Format | Catalog No. |
|---|---|---|
| Chromeo™ 488 Goat anti-Mouse IgG | 1 mg | 15031 |
| Chromeo™ 488 Goat anti-Rabbit IgG | 1 mg | 15041 |
| Chromeo™ 494 Goat anti-Rabbit IgG | 1 mg | 15042 |
| Chromeo™ 546 Goat anti-Mouse IgG | 1 mg | 15033 |
| Chromeo™ 546 Goat anti-Rabbit IgG | 1 mg | 15043 |
| Chromeo™ 642 Goat anti-Mouse IgG | 1 mg | 15034 |
| Chromeo™ 642 Goat anti-Rabbit IgG | 1 mg | 15044 |
| ATTO 594 Goat anti-Mouse IgG | 250 µl | 15037 |
| ATTO 594 Goat anti-Rabbit IgG | 250 µl | 15047 |
| ATTO 647N (STED) Goat anti-Mouse IgG | 250 µl | 15038 |
| ATTO 647N (STED) Goat anti-Rabbit IgG | 250 µl | 15048 |
| ATTO 655N (STED) Goat anti-Mouse IgG | 250 µl | 15039 |
| ATTO 655N (STED) Goat anti-Rabbit IgG | 250 µl | 15049 |

| Fluorescent Protein Labeling | Format | Catalog No. |
|---|---|---|
| LigandLink™ pLL-1 Kit | 1 kit | 34001 |
| LigandLink™ pLL-1-NFκB p65 Kit | 1 kit | 34004 |
| LigandLink™ pLL-1-p53 Kit | 1 kit | 34005 |
| LigandLink™ pLL-1-STAT1 Kit | 1 kit | 34006 |
| LigandLink™ Fluorescein Label | 300 rxns | 34101 |
| LigandLink™ Hexachlorofluorescein Label | 300 rxns | 34104 |

## Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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