HYDROGEN PEROXIDE (MEGAPLEX RED)

ASSAY PROTOCOL

K-MRH2O2

01/22

(1000 Microplate Assays per Kit) or
(100 Manual Assays per Kit) or
(400 Auto-Analyser Assays per Kit)

NEOGEN | Megazyme

CONTENTS:

INTRODUCTION:

Hydrogen peroxide (H_2O_2) is the simplest peroxide (a chemical with an oxygen-oxygen single bond). H_2O_2 is used as a chemical intermediate, as a bleaching agent in the paper and pulp and textile industries, to sanitise milk handling equipment in dairy industries and in water treatment operations.

Megaplex Red (also known as 10-acetyl-3,7-dihydroxyphenoxazine) is a highly sensitive and stable probe for H_2O_2. In the presence of horseradish peroxidase (HRP), Megaplex Red reacts with hydrogen peroxide to produce the red-fluorescent oxidation product, resorufin. Resorufin has a fluorometric (excitation and emission maxima of 571 and 585 nm respectively) and colourimetric (absorbance maximum at ~ 570 nm) signal allowing for the use of both fluorometric and colourimetric detection methods.

The Megazyme Hydrogen Peroxide Assay Kit (Megaplex Red) (**K-MRH2O2**) is a simple, user friendly method capable of detecting H_2O_2 in various samples. This kit can also be used to measure enzymatic activity in oxidase-mediated reactions that produce H_2O_2 (subject to end user development).

PRINCIPLE:

The H_2O_2 quantification assay is based on the HRP mediated oxidation of Megaplex Red. Briefly, in the presence of HRP and H_2O_2 the colourless Megaplex red probe is oxidised in a 1:1 stoichiometry to a coloured product (resorufin) that can be measured using a fluorometer equipped for excitation in the range of 530-560 nm and emission at ~ 590 nm or using a spectrophotometer set at 570 nm. H_2O_2 in the sample is then quantified using a calibration curve in fluouresence mode or a single-point standard of known H_2O_2 concentration in absorbance mode.

$$H_2O_2 + \text{Megaplex Red} \xrightarrow{\text{HRP}} H_2O + \text{resorufin (coloured)}$$

This principle is shown in more detail in Figure 1 (page 13).

SENSITIVITY, LINEARITY AND PRECISION:

In fluorescence mode, the detection limit is 0.0034 ug/mL. As fluorescence values may vary greatly between instruments, the limit of detection is calculated using the lowest value from the linear range of the assay in fluorescence mode, using the recommended sample volume of 0.01 mL. The assay is linear over the range of 0.0034-0.17 µg/mL (0.1 to 5 µM) of H_2O_2 per assay in fluorescence mode.

In absorbance mode, the detection limit is 0.25 µg/mL, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 0.4 mL. The assay is linear over the range of 0.034-0.68 µg/mL (1 to 20 µM) of H_2O_2 per assay in absorbance mode.

Linearity graphs for both detection methods are shown in Figures 2 and 4 (pages 13 and 14).

A direct comparison of the Megaplex Red H_2O_2 assay to a competitor red-fluorescent oxidation product H_2O_2 assay in fluorescence mode is shown in Figure 3 (page 14). This comparison shows that the Megaplex Red assay is linear over an extended range versus the competitor's red-fluorescent oxidation product assay.

INTERFERENCE:

Interference caused by the sample matrix can be identified by performing recovery experiments, i.e. by adding a known amount of the H_2O_2 standard to the sample in the test. Quantitative recovery of this standard would be expected.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 1000 assays in microplate format (or 100 assays in manual format or 400 assays in auto-analyser format) are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Buffer concentrate (20 mL, pH 7.4) plus sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: Megaplex Red, lyophilised powder.
Stable for > 2 years below -10°C.

Bottle 3: Dimethyl sulfoxide (DMSO) (0.6 mL).
Stable for > 2 years at room temperature.

Bottle 4: Horseradish peroxidase (HRP) (0.4 mL).
Stable for > 2 years below -10°C.

Bottle 5: Hydrogen peroxide standard solution (**~ 3%,** 0.5 mL). See individual component label for exact concentration.
Stable for > 2 years; store sealed at **4°C.**

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Visually inspect bottle 1 for crystallisation before use. If the buffer has crystallised, heat to ~ 40°C for 10 min and mix by inversion until redissolved. Dilute the contents of bottle 1 before use by adding 4 mL of buffer to 36 mL of dH_2O (this is the **Assay Buffer**). This is sufficient for ~ 200 microplate or ~ 20 manual assays with an excess for standard and sample dilution.
   Stable for > 3 months at 4°C.
2. Tap the vial on a solid surface prior to opening to ensure that all solid falls to the bottom of the tube. Dissolve the contents by adding 0.55 mL of bottle 3 (DMSO) into the vial. Recap the tube and mix thoroughly. This is the **Megaplex Red solution.** Split into aliquots of ~ 0.1 mL before storage to avoid repeated freeze thaw cycles.
   Stable for > 2 years below -10°C.
3. Use the contents of bottle 3 as supplied. DMSO has a high freezing point and the contents may freeze if room temperature falls below ~ 20°C. Ensure the DMSO is in liquid form before use by warming gently if necessary.
4. Use the contents of bottle 4 as supplied. Store the bottle in an upright position.
   Stable for > 2 years below -10°C.
5. **Dilute the H_2O_2 standard before use.** Consult the label of the H_2O_2 standard to determine the exact percentage (% w/v) of the solution prior to dilution, as the concentration may vary by lot. A guide dilution procedure is given in each assay protocol section.

NOTE: The appropriate H_2O_2 standard solution should be tested with every set of assays as it is used in the calculation of H_2O_2 concentration in the sample analysed.

PREPARATION OF WORKING SOLUTION:

Pipette the following into a ~ 13 mL polypropylene tube. This volume is sufficient for ~ 200 microplate assays or ~ 20 manual cuvette assays.

Component	Volume
Assay Buffer Megaplex red bottle 4 (HRP)	9.825 mL 0.100 mL 0.075 mL
Total volume	10 mL
Mix well. Make fresh on day of use. Do not store.	

EQUIPMENT (RECOMMENDED):

1. Polypropylene tubes (1.5 mL and ~ 20 mL capacity).
2. Microplate suitable for fluorometric detection or disposable plastic cuvettes (1 cm light path, 1.5 mL).
3. Micro-pipettors, e.g. Gilson Pipetman® (200 µL and 1000 µL).
4. Positive displacement pipettor, e.g. Eppendorf Multipette® with 25 mL Combitip® [to dispense 1 mL aliquots of bottle 1, concentrated buffer solution].
5. 20 mL graduated cylinder.
6. Fluorometer equipped for ~ 530-560 nm (excitation) and ~ 590 nm (emission) or Spectrophotometer set at 570 nm, and temperature controlled to 25°C.
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).

A. MICROPLATE ASSAY PROCEDURE (FLUORESCENCE MODE):

NOTE:
For each set of samples analysed a calibration curve must be performed concurrently in fluorescence mode using the same batch of reagents.

PREPARATION OF H_2O_2 CALIBRATION CURVE:

For fluorescence mode, dilute the H_2O_2 standard (bottle 5) to 0.68 µg/mL (20 µM). Calculate the required dilution factor based on the exact stock concentration as per the bottle 5 label. This is the **"diluted standard"**. An example of appropriate dilution for a **3% w/v** solution is outlined below.

Example:

Where bottle 5 contains a stated H_2O_2 concentration of 3% w/v, dilute by dispensing 22.7 µL of bottle 5 into a polypropylene tube. Add 977.3 µL of Assay Buffer. Mix well by vortexing. Perform a serial dilution by pipetting 10 µL of this solution into a second polypropylene tube and adding 9.99 mL of Assay Buffer. Mix well by vortexing. This is the **diluted standard** (0.68 µg/mL H_2O_2).

Prepare the H_2O_2 solutions as described in the table below (standards 1-5, 0.0034-0.17 µg/mL of H_2O_2 in the assay) for use in the fluorescence assay procedure as described below. Use fresh on day of preparation, these standards cannot be stored for future use.

Pipette into 13 mL polypropylene tubes	**STD 1** 0.0034 µg per mL	**STD 2** 0.034 µg per mL	**STD 3** 0.08 µg per mL	**STD 4** 0.114 µg per mL	**STD 5** 0.17 µg per mL
Assay Buffer **diluted standard**	1.47 mL 0.03 mL	1.2 mL 0.3 mL	0.8 mL 0.7 mL	0.5 mL 1.0 mL	- 1.5 mL
total volume	1.5 mL	1.5 mL	1.5 mL	1.5 mL	1.5 mL

ASSAY PROCEDURE:

Detection method: Fluorescence
Excitation: 530-560 nm
Emission: ~ 590 nm
Microplate: 96-well (e.g. black, flat-bottomed, polystyrene)
Temperature: ~ 25°C
Final volume: 0.10 mL
Linearity: 0.0034-0.17 µg/mL of H_2O_2 per assay (0.1-5 µM in-assay)

Pipette into wells	Blank	Sample	Standard
Assay Buffer	0.050 mL	0.040 mL	0.025 mL
sample	-	0.010 mL	-
standards 1 - 5	-	-	0.025 mL
Mix*, allow the reaction to equilibrate to temperature for 5 min and start the reaction by addition of:			
working solution	0.05 mL	0.05 mL	0.05 mL
Mix* and read the absorbance of the solutions (Abs) after exactly 30 min.			

* for example using microplate shaker, shake function on a microplate reader or repeated aspiration (e.g. using a pipettor set at 50-100 µL volume).

CALCULATION:

1. Determine the average of the duplicate readings for the blank, H_2O_2 standards and samples.
2. Correct for background fluoresence by subtracting the average of the blank values from the H_2O_2 standard values thereby obtaining $Standard_{cor}$.
3. Plot a calibration curve showing the corrected fluorescence values ($Standard_{cor}$) on the X-axis against the concentration of H_2O_2 [µg/mL in-assay] on the Y-axis using a suitable software such as Excel. A new calibration curve must be completed for each assay.
4. Calculate the linear trendline and the equation of the line based on your calibration curve data.
5. Correct for background fluoresence in the samples of interest by subtracting the average of the blank values from the average sample values thereby obtaining $Sample_{cor}$.
6. Extrapolate the concentration of the corrected sample using the following equation;

$$y = \text{Slope (m)} \times Sample_{cor} + b \quad [\mu g/mL \text{ in-assay}]$$

The concentration of H_2O_2 in the sample is calculated as follows:

$$c = \frac{y \times 0.1 \times F}{0.01} \quad [\mu g/mL]$$

where:

y	=	µg/mL in-assay calculated from the standard curve
0.1	=	total assay volume [mL]
F	=	dilution of sample
0.01	=	sample volume in-assay [mL]

The content of H_2O_2 in µM can then be calculated, if required, as follows:

$$c = \mu g/mL \times 1000 \times \frac{1}{34.015} \quad [\mu M]$$

where:

µg/mL	=	concentration of H_2O_2 in the sample
1000	=	conversion from mL to L
34.015	=	Molecular weight of H_2O_2 in g/mol

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears in the Megazyme web site (www.megazyme.com).

Example:

The linear trendline for the sample data in Figure 1 (Page 13) of this assay protocol gives the following equation:

$$y = 0.0004x - 0.0005 \text{ (in the form } y = mx + b)$$

If the $Sample_{cor}$ value in this analysis is 300 RFU for an undiluted sample then the concentration of H_2O_2 in the assay can be calculated as follows:

$$y = 0.0004 \times 300 - 0.0005 = 0.1195 \text{ µg/mL in-assay}$$

The concentration of H_2O_2 in the sample is calculated as follows:

$$c = \frac{0.1195 \times 0.1 \times 1}{0.01} = 1.195 \text{ µg/mL}$$

The content of H_2O_2 in µM can then be calculated, if required, as follows:

$$c = 1.195 \times 1000 \times \frac{1}{34.015} = 35.13 \text{ µM}$$

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears in the Megazyme web site (www.megazyme.com).

B. MICROPLATE ASSAY PROCEDURE (ABSORBANCE MODE):

PREPARATION OF H_2O_2 STANDARD:

For absorbance mode, prepare a single point standard by diluting the H_2O_2 standard (bottle 5) to 6.8 µg/mL (200 µM). Calculate the required dilution factor based on the exact stock concentration as per the bottle 5 label. This is the **"diluted standard"**. An example of an appropriate dilution for a **3% w/v** solution is outlined below.

Example:

Where bottle 5 contains a stated H_2O_2 concentration of 3% w/v dilute by dispensing 22.7 µL of bottle 5 into a polypropylene tube. Add 977.3 µL of Assay Buffer. Mix well by vortexing. Perform a serial dilution by pipetting 10 µL of this solution into a second polypropylene tube and adding 990 µL of Assay Buffer. Mix well by vortexing. This is the **diluted standard** (6.8 µg/mL H_2O_2).

ASSAY PROCEDURE:

Detection method: Absorbance
Wavelength: 570 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 25°C
Final volume: 0.10 mL
Linearity: 0.03-0.68 µg/mL of H_2O_2 per assay (1-20 µM in-assay)

Pipette into cuvettes	Blank	Sample	Standard
Assay Buffer	0.050 mL	0.040 mL	0.045 mL
sample	-	0.010 mL	-
diluted standard	-	-	0.005 mL
Mix*, allow the reaction to equilibrate to temperature for 5 min and start the reaction by addition of:			
working solution	0.05 mL	0.05 mL	0.05 mL
Mix* and read the absorbance of the solutions (A_1) at the end of the reaction (~ 30 min).			

* the sample volume in the test can be increased to 0.04 mL if an increase in sensitivity is required. If the sample volume is increased, the volume of solution 1 (Assay Buffer) should be reduced proportionately.
** for example using microplate shaker, shake function on a microplate reader or repeated aspiration (e.g. using a pipette set at 50-100 µL volume).

CALCULATION:

Determine the absorbance difference between the absorbance of the standard and of the blank ($Abs_{standard}$ - Abs_{blank}) after 30 min, thereby obtaining $\Delta Abs_{standard}$.

Calculate **M** as follows:

$$\mathbf{M} = \frac{H_2O_2 \text{ (µg/mL in-assay)}}{\Delta Abs_{standard}}$$

Determine the absorbance difference between the absorbance of the sample and of the blank (Abs_{sample} - Abs_{blank}) after 30 min, thereby obtaining ΔAbs_{sample}.

The concentration of H_2O_2 can then be calculated as follows:

$$\mathbf{c} = \Delta Abs_{sample} \times \mathbf{M} \times \frac{V_1}{V_2} \times F \quad [\mu g/mL]$$

where:

ΔAbs_{sample}	=	Abs_{sample} - Abs_{blank}
M	=	value of H_2O_2 standard [µg/$\Delta Abs_{standard}$]
V_1	=	total assay volume [mL]
F	=	dilution of sample
V_2	=	sample volume in assay [mL]

The content of H_2O_2 in µM can then be calculated, if required, as follows:

$$\mathbf{c} = \mu g/mL \times 1000 \times \frac{1}{34.015} \quad [\mu M]$$

where:

µg/mL	=	concentration of H_2O_2 in the sample
1000	=	conversion from mL to L
34.015	=	Molecular weight of H_2O_2 in g/mol

Example:

Where the 6.8 µg/mL diluted standard is prepared as decribed in the assay procedure and 0.005 mL of this is used in a final assay volume of 0.1 mL the amount of H_2O_2 in assay is 0.34 µg/mL. If, for example, the $\Delta Abs_{standard}$ measured in the assay is 0.650, then the "M" value can be calculated as follows:

$$\mathbf{M} = \frac{0.34}{0.650} = 0.52$$

If the ΔAbs_{sample} measured in the assay for an undiluted sample is 0.700 then the concentration of H_2O_2 is calculated as follows:

$$c = 0.700 \times 0.52 \times \frac{0.1}{0.01} \times 1 = 3.64 \text{ µg/mL}$$

The content of H_2O_2 in µM can then be calculated, if required, as follows:

$$c = 3.64 \times 1000 \times \frac{1}{34.015} = 107 \text{ µM}$$

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears in the Megazyme web site (www.megazyme.com).

C. MANUAL ASSAY PROCEDURE (ABSORBANCE MODE):

Prepare a 6.8 µg/mL (200 µM) H_2O_2 diluted standard solution as described on page 7 in this assay protocol.

ASSAY PROCEDURE:

Detection method: Absorbance
Wavelength: 570 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: 25°C
Final volume: 1.0 mL
Sample solution: 0.034-0.68 µg/mL of H_2O_2 per assay (1-20 µM in-assay)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample	Standard
assay buffer	0.50 mL	0.40 mL	0.45 mL
sample	-	0.10 mL*	-
diluted standard	-	-	0.05 mL
Mix*, allow the reaction to equilibrate to temperature for 5 min and start the reaction by addition of:			
working solution	0.50 mL	0.50 mL	0.50 mL
Mix** and read the absorbance of the solutions (Abs) after exactly 30 min.			

* the sample volume in the test can be increased to 0.4 mL if an increase in sensitivity is required. If the sample volume is increased, the volume of solution 1 (Assay Buffer) should be reduced proportionately.
** for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

CALCULATION:

Determine the absorbance difference between the absorbance of the standard and of the blank ($Abs_{standard}$ - Abs_{blank}) after 30 min, thereby obtaining $\Delta Abs_{standard}$.

Calculate **M** as follows:

$$\mathbf{M} = \frac{H_2O_2\ (\mu g/mL\ \text{in-assay})}{\Delta Abs_{standard}}$$

Determine the absorbance difference between the absorbance of the sample and of the blank (Abs_{sample} - Abs_{blank}) after 30 min, thereby obtaining ΔAbs_{sample}.

The concentration of H_2O_2 can then be calculated as follows:

$$\mathbf{c} = \Delta Abs_{sample} \times \mathbf{M} \times \frac{V_1}{V_2} \times F \qquad [\mu g/mL]$$

where:

ΔAbs_{sample}	=	Abs_{sample} - Abs_{blank}
M	=	value of H_2O_2 standard [$\mu g/\Delta Abs_{standard}$]
V_1	=	total assay volume [mL]
F	=	dilution of sample
V_2	=	sample volume in assay [mL]

The content of H_2O_2 in µM can then be calculated, if required, as follows:

$$\mathbf{c} = \mu g/mL \times 1000 \times \frac{1}{34.015} \qquad [\mu M]$$

where:

µg/mL	=	concentration of H_2O_2 in the sample
1000	=	conversion from mL to L
34.015	=	Molecular weight of H_2O_2 in g/mol

Example:

Where the 6.8 µg/mL diluted standard is prepared as decribed in the assay procedure and 0.05 mL of this is used in the assay the amount of H_2O_2 in assay is 0.34 µg/mL. If, for example, the $\Delta Abs_{standard}$ measured in the assay is 0.650, then the "M" value can be calculated as follows:

$$\mathbf{M} = \frac{0.34}{0.650} = 0.52$$

If the ΔAbs_{sample} measured in the assay for an undiluted sample is 0.700 then the concentration of H_2O_2 is calculated as follows:

$$\mathbf{c} = 0.700 \times 0.52 \times \frac{1.0}{0.1} \times 1 = 3.64 \text{ µg/mL}$$

The content of H_2O_2 in µM can then be calculated, if required, as follows:

$$\mathbf{c} = 3.64 \times 1000 \times \frac{1}{34.015} = 107 \text{ µM}$$

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears in the Megazyme web site (www.megazyme.com).

D. AUTO-ANALYSER ASSAY PROCEDURE:

NOTE:
For each set of samples tested in the Auto-Analyser format either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.

Reagent preparation for ~ 100 assays is performed as follows:

R1: Assay buffer, 10 mL (see preparation, page 3)

R2: Working solution, 10 mL (see preparation, page 3)

H_2O_2 standard: Prepare a diluted H_2O_2 standard as decsribed on page 7 in this assay protocol.

EXAMPLE METHOD:

R1:	0.1 mL
Sample:	0.025 mL
R2:	0.125 mL
Reaction time:	30 min at 25°C
Wavelength:	570 nm
Prepared reagent stability:	R1 is stable for ~ 3 months stored at 4°C. Prepare R2 and H_2O_2 standard freshly on the day of assay.
Reaction direction:	Increase
Linearity:	up to 9.0 µg/mL of H_2O_2 using 0.025 mL sample volume

SAMPLE PREPARATION:

Fluorescence mode:

In fluorescence mode, the amount of H_2O_2 present in the test should range between 0.00034-0.017 µg per assay (0.1-5 µM in-assay concentration). The original sample solution must therefore be diluted sufficiently to yield a H_2O_2 concentration between 0.034-1.7 µg/mL (1-50 µM) when using the recommended assay volume of 0.01 mL. In order to achieve sufficiently accurate results the value for the sample in relative fluorescence units (RFU) should not exceed the value achieved for STD 5 (0.17 µg/mL in-assay) in the calibration curve. Recommended dilutions of sample are described in the table below.

H_2O_2 (µg/mL)	H_2O_2 (µM)	Dilution with dH_2O	Dilution factor (F)
< 1.7	< 100	No dilution	1
1.7-17	100-1000	1 + 9	10
17-170	1000-10,000	1 + 99	100
> 170	> 10,000	1 + 999	1000

If the RFU value is lower than STD 1 (0.0034 µg/mL in-assay) in the calibration curve, weigh out more sample or dilute less strongly.

Absorbance mode:

In absorbance mode (microplate), the amount of H_2O_2 present in the test (i.e. in the 0.01 mL of sample being analysed) should range between 0.003-0.068 µg per assay (1-20 µM in-assay concentration). In absorbance mode (manual) the amount of H_2O_2 present in the test (i.e. in the 0.1 mL of sample being analysed) should range between 0.034-0.68 µg per assay (1-20 µM in-assay concentration). The original sample solution must therefore be diluted sufficiently to yield a H_2O_2 concentration between 0.34-6.8 µg/mL (10-200 µM) before analysis. In order to achieve sufficiently accurate results, the value of ΔAbs_{sample} should as a rule be at least 0.1 absorbance units and not more than 2.0 absorbance units in absorbance mode. Recommended dilutions of sample are described in the table below.

H_2O_2 (µg/mL)	H_2O_2 (µM)	Dilution with dH_2O	Dilution factor (F)
< 6.8	< 0.5	No dilution	1
6.8-68	0.5-5	1 + 9	10
68-680	5-50	1 + 99	100
> 680	> 50	1 + 999	1000

If the value of Abs_{sample} is too low (e.g. < 0.1), weigh out more sample or dilute less strongly.

APPENDIX:

A. Assay Principle - H_2O_2 detection

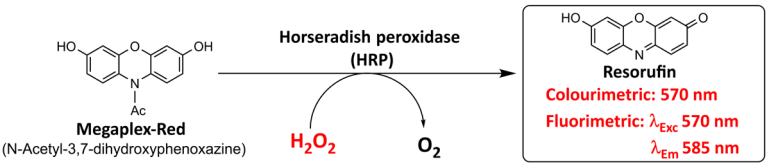

Figure 1. Theoretical basis of the H_2O_2 detection assay.

B. Linear range of assay

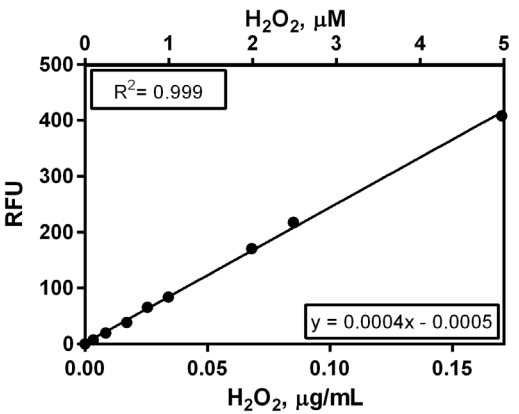

Figure 2. Linearity of the H_2O_2 assay in fluorescence mode. Fluorescence was measured with a fluorescence microplate reader using excitation at 530 nm and emission at 590 nm wavelengths. Note that a new calibration curve must be completed for each batch of samples. This data is provided for demonstrative purposes only.

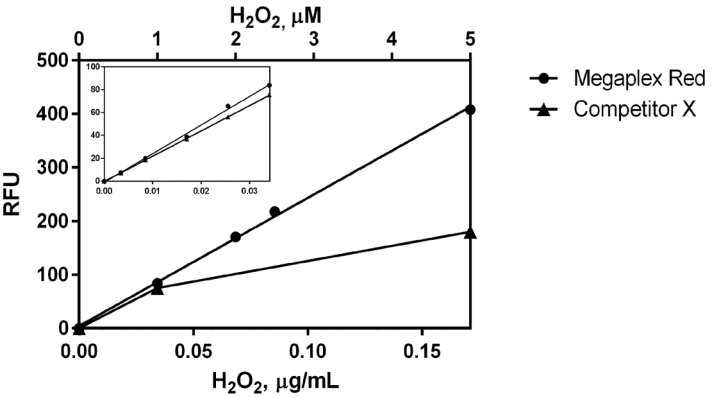

Figure 3. Comparison of linearity of the H_2O_2 assay in fluorescence mode versus a comparable competitor red-fluorescent assay. The Megaplex Red assay is linear over an extended range. Fluorescence was measured with a fluorescence microplate reader using excitation at 530 nm and emission at 590 nm wavelengths.

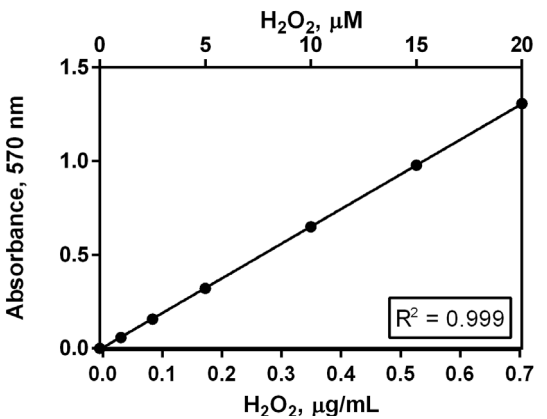

Figure 4. Linearity of the H_2O_2 assay in absorbance mode.

C. Precision of the assay

Analyte	n	µg H_2O_2 per test	Mean, RFU H_2O_2	Standard Deviation	% CV
H_2O_2	10	0.00034	7.142	0.278	3.9
	10	0.0017	35.520	2.970	8.4
	10	0.0034	80.180	6.081	7.6
	10	0.0085	189.944	3.010	1.6
	10	0.0170	381.247	8.415	2.2

Figure 5. Intermediate precision values in microplate fluoresence mode obtained using a range of H_2O_2 standards in the assay kit.

Analyte	n	µg H_2O_2 per test	Mean, Abs H_2O_2	Standard Deviation	% CV
H_2O_2	23	0.170	0.339	0.024	7.2
	24	0.340	0.705	0.043	6.1
	24	0.680	1.413	0.049	3.5

Figure 6. Intermediate precision values in manual absorbance mode obtained using a range of H_2O_2 standards in the assay kit.

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