# Data Sheet

## Eucodis Peroxidase Kit

| Cat. No. | Sales unit |
|---|---|
| EP Kit | 50 mg - 1 g |

For research use only.
Store at -20°C.

### Application

Eucodis Peroxidases can be utilized as enzymes catalyzing e.g. aromatic ring hydroxylation, epoxidation, halogenation, N- or S-oxidation, ether cleavage and alcohol/aldehyde oxidation reactions.

### Form

8 vials
Powder, slightly brown-red, lyophilized

### Specifications

| | |
|---|---|
| Name | Eucodis Peroxidase Kit |
| E.C. | 1.11.1.7 |
| Origin | bacterial and fungal |
| Source | recombinant |
| pH Optimum | 5-8 |
| pH Stability | 5-8 |
| Temp. Optimum | 20-40°C |

### Reconstitution

Eucodis Peroxidases are soluble in water and should be reconstituted in $dH_2O$ to a final concentration of 20 mg/ml in 50 mM phosphate buffer.

Before use any insoluble material may be removed by centrifugation.

### Function

Peroxidases in this kit belong to the class of the heme-family peroxidases (heme-thiolate, peroxidockerin, DyP-type, hybrid, versatile, tyrosine-ring hydroxylase and chlorite dismutase).

Peroxidases can be used in any oxygenation reactions using hydrogen peroxide as a co-substrate with heme as the prosthetic group. Broad range of industrial applications include waste water treatment (oxidation of chemical effluents from industrials waste), fabric industry (colorizing and decolorizing dyes), food and flavor industry (synthesis of aromatics), cosmetics (perfumes), green plastics industry (dicarboxylic acid synthesis) as well as pharmaceutical applications (API synthesis, e.g. antibiotics synthesis).

The Eucodis Peroxidase Kit contains each of the following peroxidases:

| Eucodis Peroxidase Kit | | | |
|---|---|---|---|
| EP001 | EP003 | EP004 | EP009 |
| EP010 | EP012 | EP013 | EP014 |

## General Recommendations for Use

Reactions are performed at room temperature for desired time periods (overnight incubation period is a good starting point).

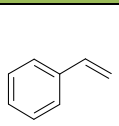
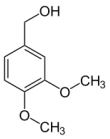
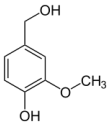
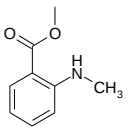
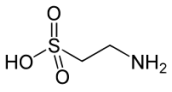
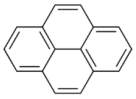
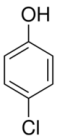
For water-soluble substrates:

- 10 mM t-butyl hydroperoxide or 1 mM H2O2 as co-substrate
- 1 mM substrate
- 0.1-1 mg/ml peroxidase lyophilisate
- 20 mM phosphate buffer, pH 5.0 shows best activity in most substrates tested.

For sparingly water-soluble substrates:

- 1-10 mM t-butyl hydroperoxide or 1 mM H2O2 as co-substrate
- 100-500 µM substrate in Dimethyl formamide or Dimethyl sulfoxide or Tetrahydrofuran
- 0.1-1 mg/ml peroxidase lyophilisate
- Organic solvents: we recommend using Hexane, Dimethyl formamide (DMF), Dimethyl sulfoxide (DMSO) or Tetrahydrofuran (THF) at concentrations up to 20% of total reaction volume and neutral pH.

Model reactions performed by Eucodis:

| Peroxidase | Model Reactions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Epoxidation | Oxidation of alcohols | | N-Demethylation | S-Oxidation | Halogenation | | Aromatic ring hydroxylation | Dehalogenation |
| | Styrene | Veratryl Alcohol | Vanillyl alcohol | N-methyl anthranilate | Thioanisole | Taurin/ $Cl^-$ | Taurin/ $Br^-$ | Pyrene | 4-chlorophenol |
| |  |  |  |  |  |  | |  |  |
| EP001 | - | + | + | - | + | + | + | - | + |
| EP003 | - | + | + | - | - | + | + | - | - |
| EP004 | - | + | + | - | + | - | - | + | - |
| EP009 | - | - | + | - | + | - | - | - | - |
| EP010 | - | + | + | - | + | - | - | + | - |
| EP012 | + | + | + | + | + | - | - | - | - |
| EP013 | + | + | + | + | - | - | - | + | - |
| EP014 | + | + | + | - | - | - | - | - | - |

**Peroxidase Microplate Assay Setup:**

The reaction is performed in a final volume of 200 µl by mixing 100 µl enzyme solution and 100 µl substrate solution.

Prepare a dilution series of the enzyme starting at 10 mg/ml down to approx. 0.1 mg/ml in 1:3 steps in 50 mM phosphate buffer, pH 5.0. Add 100µl of each enzyme dilution per well into a 96-well plate.
Then, prepare the substrate solution containing 2 mM ABTS and 20 mM $H_2O_2$, or 16 mM Guaiacol and 16 mM $H_2O_2$, respectively, in 50 mM phosphate buffer, pH 5.0.

After all enzyme dilutions are layed out in the 96-well plate, add 100 µl substrate solution into each well, mix, and immediately start the measurement in a microplate reader.

**Guaiacol Assay:**

Recommended conditions for the Guaiacol assay are (final concentrations):

- 200 µl assay volume
- 50 mM phosphate buffer pH 5.0
- 8 mM Guaiacol
- 8 mM $H_2O_2$
- 0.1-5 mg/ml peroxidase lyophilisate

Determine increase in absorbance at 470 nm ($\varepsilon_{470nm}$ = 26000 $M^{-1}$ $cm^{-1}$) over a suitable time period.

**ABTS Assay:**

Recommended conditions for the ABTS assay are (final concentrations):

- 200 µl assay volume
- 50 mM phosphate buffer pH 5.0
- 1 mM ABTS
- 10 mM $H_2O_2$
- 0.1-5 mg/ml peroxidase lyophilisate

Determine increase in absorbance at 405 nm ($\varepsilon_{405nm}$ = 36000 $M^{-1}$ $cm^{-1}$) over a suitable time period.

Example ABTS Assay:

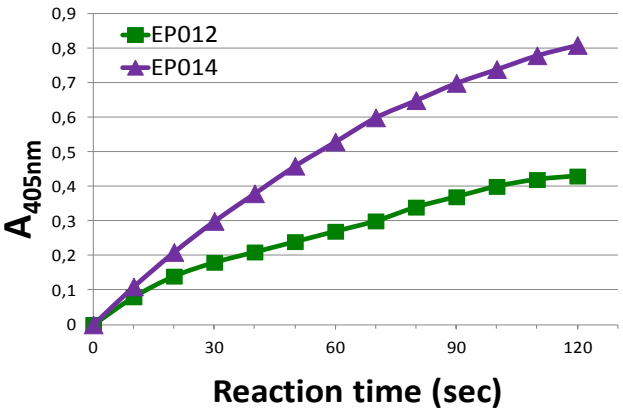



**Figure 1: ABTS activity assay for Eucodis Peroxidases EP012 and EP014**. Activity was measured over a 2 min interval at 405nm.

**Characterization Results:**

The Eucodis Peroxidases have been selected to cover a broad spectrum of subfamilies, and their natural substrates are often unknown. Therefore, general activities of the peroxidases cannot be deduced from detected activities with the standard substrates ABTS or Guaiacol, and low activities with these substrates may only reflect their specificity for other classes of substrates. We therefore always recommend testing all enzymes in the screening kit against your substrate of choice.

| | ABTS | Guaiacol | Peroxidase features |
|---|---|---|---|
| **EP001** | ++ | ++ | Conversion of essential oil ingredient to colored product, fatty acid epoxidation |
| **EP003** | weak | + | Fatty acid epoxidation |
| **EP004** | weak | weak | S-oxidation |
| **EP009** | + | no reaction detected | S-oxidation |
| **EP010** | + | + | Oxidation of pyrene |
| **EP012** | + | + | Good conversion of essential oil terpenoids (oxygenation, epoxidation and oxidations) with different specificities than EP013, styrene epoxidation |
| **EP013** | + | + | Good conversion of essential oil terpenoids (oxygenation, epoxidation and oxidations) with different specificities than EP012, styrene epoxidation |
| **EP014** | weak | weak | Fatty acid epoxidation, styrene epoxidation |