Hypoxyprobe, Inc.

121 Middlesex Turnpike

Burlington, MA 01803 USA

www.hypoxyprobe.com

Hypoxyprobe™-Overview

Hypoxyprobe™- Technology

Our **Pimonidazole HCl salt** forms adducts with thiol containing proteins at pO2 ≤ 10 mm Hg. This is considered a very low amount of oxygen and is refered to as ***hypoxic tissue***. Our various antibodies bind to the **pimonidazole HCl protein-thiol** adducts in hypoxic cells in tissues and in culture.

The **Pimonidazole free base** sold by other companies will **not work**.

There are 2 common Detection Methods of Antibody binding Pimonidazole HCl-Thiol containing proteins (under hypoxic conditions).

1 .The first method is the use of chromogen DAB for visible brown staining for use in Immunohistochemical staining, ELISA or visible microscopy.

2. The second detection method involves the use of fluorescence techniques. Fluorescence Antibodies are useful in FACS and Western Blotting as well as Fluorescence Microscopy.

Chromogen DAB Method Explained

Produces Brown Stain

A secondary antibody is added that will selectively bind the primary antibody that is in turn bound to the **reduced pimonidazole HCl protein-thiol complex**. This secondary antibody is conjugated to Horse Radish Peroxidase (HRP). Addition of the Chromogen to the bound (selectively) secondary antibody, reacts with the Horse Radish Peroxidase in the areas of tissue that were Hypoxic.

Fluorescence Techniques Explained

Produces different wavelengths of light Emmision (Fluorescence) in response to Various wavelengths of light Excitation

Again, a secondary antibody is added that will selectively bind the primary antibody that is in turn bound to the **reduced pimonidazole HCl protein thiol complex**. In this method though, instead of the HRP conjugation, the secondary antibody is conjugated to a dye that will fluoresce with its specific fluorescence wavelength(s) of Light. To get the dye to fluoresce it is excited with a specific wavelength(s) of light that is required for that dye. This Excitation wavelengths are usually, but not always, quite different than the Emmision wavelengths.

121 Middlesex Turnpike · Burlington MA · 01803 · USA · Tel 781 272 6888· Fax 781 272 9288· tlee@natural-pharma.com