

Ram Seminal Vesicle derived Microsomes Product Number: V 02 Aliquot: 5 mg Lot Number v02.060405A Storage: -80°C

- **DESCRIPTION:** Eicosanoid metabolizing enzymes such as cycloxygenases, prostaglandins, and numerous other enzymes, are microsomal membrane associated and are found highly expressed in seminal vesicle tissues. This microsomal fraction has been isolated from ram seminal vesicles with care to minimize the loss of enzymatic activity.
- **CONCENTRATION:** 5.0 mg/mL total protein using the BCA protein assay with BSA as a standard.
- **STORAGE BUFFER:** 100 mM Sodium Phosphate, 250 mM Mannitol; 1 mM PMSF; 10 mM EDTA; pH 7.8

STORAGE: –80°C AVOID MULTIPLE FREEZE-THAW CYCLES.

SOURCE: Microsomal fractions were derived from frozen ram seminal vesicles that were collected and placed at -80 °C immediately after excision from post-slaughtered male ovine bucks.

SPECIFIC ACTIVITY: 169 pmol of metabolized Arachidonic Acid / μ g protein / min at 37°C ± 0.5 pmol

INDOMETHACIN INHIBITION:

APPLICATIONS:

This microsomal fractionation can be used as a source of active eicosanoid metabolizing enzymes. Many eicosanoids can be solubilized using the following protocol:

This preparation of microsomes exhibited and IC50 of ~55 nM for enzyme inhibition of Arachidonic Acid with Indomethacin.

NOTE – All steps should be performed on ice, using ice cold solutions, or at 4°C where appropriate.

- Ultracentrifuge suspended microsomes at 110,000 x g for 90 minutes and discard the resulting supernatant.
- 2) Resuspend microsome pellet in 2-4 x volume of 10 mM Tis-HCl; 0.5 mM EDTA; 1% Tween 20; pH to 8.0 using deoxygenated water with 3 x strokes of a Teflon pestle homogenizer.
- 3) Stir suspension for 45 minutes.
- 4) Ultracentrifuge the stirred suspension of microsomes at 100,000 x g for 90 minutes and collect the supernatant of solubilized eicosanoids.

The solubilized eicosanoids are now ready for further processing or aliquot and storage at -80°C.