StressMarq Biosciences INC.
PO Box 50366 Cadboro Bay
Victoria BC V8N 4G0
CANADA
Tel: +1-250-294-9065
Fax: +1-250-294-9025

StressXpress®

Saliva Sample Handling Instructions

INTRODUCTION:

The use of saliva as a relatively non-invasive sample has become widespread. However it's use suffers from a number of potential drawbacks. These are high viscosity, potential discoloration, and particles from food intake. There are also known interferences that can occur depending on the method of collection or processing. These interferences can be high pipetting variability, variable background signal in spectrophotometric signal detection and nonspecific binding.

MATERIALS NEEDED:

- 15 mL centrifuge tubes
- Plastic vials
- Protease inhibitors
- Activated Sodium Orthovanadate
- Other enzyme inhibitors as appropriate

PROCEDURE:

General

Whole saliva should be obtained at least 2 h after eating and rinsing mouth with water to avoid any food borne antigens or materials from affecting the analysis.

Activated Orthovanadate

200 mM Activated Orthovanadate should be prepared by dissolving 1.84 g of sodium orthovanadate in 45 mL of water. Adjust the pH of the solution to 10 with 1M NaOH or HCl. At pH 10 the solution should be yellow. Boil the solution until it turns colorless (approximately 10 min). All of the orthovanadate should dissolve. Cool to room temperature and readjust the pH to 10. Repeat the boiling of the solution and pH readjustment until the solution is colorless and remains at pH 10. Adjust the final volume to 50 mL with water. Store the Activated Sodium Orthovanadate in aliquots and freeze at -20°C. Use an aliquot for preparing Activated Cell Lysis Buffer and discard.

Stable Antigens

Methods of saliva collection vary widely. In our labs we ask volunteers to collect their saliva by allowing the saliva to passively flow into a 15 mL centrifuge tube. For measurements of stable antigens, such as steroids, the saliva is frozen at -20°C. Upon thawing, the saliva is centrifuged at 2,500 x g for 20 minutes and the clear supernatant is pipetted off any precipitated material. Analyze immediately or aliquot and freeze at -20°C.

Possibly Unstable Antigens

To minimize degradation of unstable antigens, keep samples on ice and immediately add enzyme inhibitors, such as, protease inhibitor cocktail (Sigma, 1 µL/mL whole saliva) and 1 mM of sodium orthovanadate for peptides and proteins, IBMX (Catalog Number P019-100MG and P019-1GM) for cyclic nucleotide measurements, or a general cyclooxygenase inhibitor, such as meclofenamic acid or indomethacin at 15 µM for prostaglandin assays, are added immediately after sample collection. All samples should be kept on ice during the process. The saliva is frozen at -20°C. Upon thawing, the saliva is centrifuged at 2,500 x g for 20 minutes and the clear supernatant is pipetted off any precipitated material. Analyze immediately or aliquot and freeze at -80°C.