DetectX®

# Saliva Sample Handling Instructions

For use with DetectX® Immunoassay and Detection Kits



## INTRODUCTION

Due to its scientifically established accuracy in hormone measurement and non-invasive sampling methods, saliva has become a widespread medium for sample analysis. In 2018, PubMed recorded 261 publications with documented saliva analysis, showing a yearly increase in both human and animal studies.

Many factors should be considered when handling saliva samples. The way saliva is processed (e.g. pooling, spiking, shipping conditions) may alter the matrix or cause artifical matrix effects not found with natural saliva. As a precaution, the storage temperature of the saliva, which varies based on the analyte, should be determined for optimum stability. For example, cortisol is stable in saliva at 4°C or room temperature for one week.

When running an immunoassay, it is typical that discolorants present in the sample will be washed out prior to signal detection readings. For activity or chemical detection assays, a pre-read of the plate may be necessary when a sample coloration is present.

Please search Pubmed (www.ncbi.nlm.nih.gov/pubmed) for recommended salivary methods for the analyte you are trying to measure.

## MATERIALS NEEDED

- 15 mL centrifuge tubes
- Plastic vials
- Protease inhibitors for protein or peptide hormones
- Other enzyme inhibitors as appropriate

## PROCEDURE

### General

Whole saliva should be collected at least 2 hours after eating. Mouth should be rinsed with water to avoid any food-borne antigens or materials that may affect analysis. Methods of saliva collection vary widely. In our labs, we ask volunteers to collect saliva by allowing the saliva to passively flow into a 15 mL centrifuge tube. For home or in-field saliva collections, we recommend a saliva collection device. Please follow the recommendations of previous studies for saliva collection.

### Stable Antigens

For measurements of stable antigens, such as steroids, the saliva is frozen at -20°C to precipitate out mucosal proteins and materials. Upon thawing, centrifuge the saliva at 2,500 x g for 20 minutes. The clear supernatant can be pipetted off of any precipitated material. Analyze immediately or aliquot and freeze at ≤ -20 °C.

### Possibly Unstable Antigens

To minimize degradation of unstable antigens, immediately add enzyme inhibitors and keep the samples on ice during the process. For peptides and proteins, add a protease inhibitor cocktail, such as from Sigma, at 1 µL/mL whole saliva and activated sodium orthovanadate at 1 mM. For cyclic nucleotides, add a phosphodiesterase inhibitor like IBMX (Catalog No. P019-100MG/-1G) at 1 mM. For prostaglandins, a general cyclooxygenase inhibitor, such as meclofenamic acid or indomethacin, can be used at 15 µM. Once inhibitors are added, samples should be frozen at -20˚C to precipitate out mucosal proteins and material. Upon thawing, centrifuge saliva at 2,500 x g for 20 minutes. The clear supernatant can be pipetted off of any precipitated material. Analyze immediately or aliquots and freeze at -80˚C.