

USER PROTOCOL



SALIVA COLLECTION KIT



IBI Catalog Number: **SK-100**

A. KIT CONTENTS

- ♦ Saliva collection funnel with cap.
- ♦ Accessory screw cap for saliva sample tube.
- ♦ 5ml tube, containing 2ml of saliva suspension solution.

B. PRODUCT OVERVIEW

- ♦ The IBI saliva collection kit is designed to easily capture and store saliva samples deposited directly from patients. This kit is optimized for epigenetics.
- ♦ This kit is manufactured by IBI *SCIENTIFIC* on behalf of Behavioral Diagnostics Inc.

C. SALIVA COLLECTION PROTOCOL

COLLECTION PRECAUTION: Do NOT eat, drink, or chew gum 30 minutes prior to giving saliva sample.

- 1.) Remove the kit contents from package.
- 2.) The collection funnel with snap-on cap should be connected to the saliva storage tube.
- 3.) If the collection funnel has disconnected from the saliva storage tube, merely press the two pieces back together.
- 4.) The outside of the saliva storage tube is graduated from 1ml to 5ml, with a line indicating 2ml that goes all the way around the tube.
- 5.) Remove the snap-on cap from the collection funnel.
- 6.) Instruct the patient to deposit saliva sample into center of the collection funnel, and fill collection tube until at least 2ml but no more than 3ml level is reached.
- 7.) Take collection funnel and tube from patient.
- 8.) Remove **green** cap from 5ml saliva suspension solution tube.
- 9.) Carefully pour the entire contents (approximately 2ml) of saliva suspension solution into the saliva storage tube.
- 10.) Remove collection funnel from saliva storage tube and discard.
- 11.) Place accessory screw cap on saliva storage tube and tighten. Shake sample tube well to properly mix contents.
- 12.) Fill out ID label and place it onto the saliva storage tube.
- 13.) Place saliva storage tube in bag and seal. Store at +4°C until DNA extraction can be performed.

*For DNA Extraction Processing, please see DNA Extraction Protocol located in the next section.

D. DNA EXTRACTION PROTOCOL

This DNA Extraction Protocol is intended for use with the collected saliva sample from the IBI *SCIENTIFIC* Saliva Collection Kit, model number **SK-100**.

ADDITIONAL ITEMS REQUIRED FOR DNA EXTRACTION PROTOCOL

- 15ml conical centrifuge tube
 - 2ml centrifuge tubes
 - Proteinase K Solution - 20mg/ml (IBI Item# IB05406)
 - 5M, C₂H₇NO₂ (Ammonium Acetate)
 - Isopropanol (IBI Item# IB15730 and IB15735)
 - 70% Ethanol
 - TE Buffer (10mM Tris + 1mM EDTA) pH 8.0
- 1.) Transfer the saliva sample from the IBI saliva collection kit tube to a 15ml conical centrifuge tube (approximately 4ml) and vortex for 10 seconds to ensure complete mixing of the sample.
 - 2.) Add 40µl of Proteinase K Solution (20mg/ml), IBI Item# IB05406. Vortex for 10 seconds and incubate for 3 hours at 55°C.
 - 3.) Cool the 4ml sample to room temperature, then add 2ml of 5M C₂H₇NO₂ (Ammonium Acetate). Vortex briefly to ensure full mixing, then split sample into 3 separate 2ml microcentrifuge tubes. Spin each tube at 15,000 x G for three minutes at room temperature (or slightly cooler).
 - 4.) Harvest the supernatants and recombine them into one 15ml tube. Discard the tubes containing the precipitated proteins. Add 4ml of ice cold isopropanol to the 15ml tube already containing the combined supernatants and shake vigorously for 5 seconds. Next, spin at 3,000 x G for 10 minutes.
 - 5.) Pour off the supernatant, being careful not to disturb the DNA pellet. Wash the DNA pellet with cold 70% ethanol (Do NOT use 100% ethanol!) to remove the isopropanol. Air dry at room temperature. Do NOT use vacuum, heat, or fan.
 - 6.) Re-suspend the DNA over night with 500µl of TE Buffer (10mM Tris, 1mM EDTA, pH 8.0). The resulting DNA can be quantified with conventional spectrophotometric methods, then stored at -20°C until use.

Optional Step: Although the resulting DNA is suitable for nearly all purposes, the small amounts of protein found in saliva DNA may interfere with some sensitive procedures, such as construction of DNA libraries. These residual proteins can be removed by extraction using 1ml of chloroform (IBI Item# IB05040). To accomplish this, add 1ml of chloroform to a 2ml microcentrifuge tube, vortex for 5 seconds, then spin at 15,000 x G for 1 minute. Remove the top aqueous layer with a pipette and store at -20°C until use.

E. ORDERING INFORMATION

The IBI Saliva Collection Kit is factory packaged, sealed, and ready for use. It is available in a wide range of quantities including a single kit up to packs of 50. See full product listing below:

- **SK-100** - IBI Saliva Collection Kit - 1/Pack
- **SK-110** - IBI Saliva Collection Kit - 10/Pack
- **SK-125** - IBI Saliva Collection Kit - 25/Pack
- **SK-150** - IBI Saliva Collection Kit - 50/Pack



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