Dear Participant,

Once you have taken your saliva kit home, please open the box labeled “Start of Study” and put the 2 included ice bricks and the Therapak gel pack in your freezer. Be sure the gel pack is folded in half the way you received it and place it in a flat position in your freezer. The ice bricks and gel pack should remain in the freezer until you have finished collecting your samples for the first 3 days so they will be frozen when you are ready to ship the samples to our lab. Please note that there is bubble wrap in your kit. Do not throw away the bubble wrap. You will need to use the bubble wrap when you ship your samples.

You should begin collecting your saliva samples the morning after you have received these materials. You will need to collect your saliva three times each day for three days in a row (see saliva instructions below). Each sample should be stored inside its tube in the Ziploc bag in which it came. Store the collected samples inside your freezer until all nine samples have been collected and you are ready to pack the shipping container to return to us (see shipping instructions included separately in your kit). This entire process of collecting and shipping samples will be repeated after your 12-week follow-up. The box labeled “End of Study” should only be opened after the 12-week follow-up visit to the clinic when you are ready to start collecting the last set of samples. Remember that the ice bricks and gel pack should be placed in your freezer on the first day when you open the kit so they will be frozen when it is time to pack the kit to ship it to us.

When to Collect Your Saliva

You should begin collecting your saliva samples the morning after you have received these materials. You will need to collect your saliva three times each day for three days in a row. Each day, saliva should be collected:

1. When you wake up and before you brush your teeth
2. 30 minutes after you wake up
3. At bedtime

You will again begin collecting your saliva samples the day following your 12-week follow-up visit to the clinic. If you forget to collect a sample at the right time, please collect the sample as soon as you remember and write down the actual time that you collected it.
Guidelines for Collecting Saliva

For 30 minutes before collecting your saliva, please be sure that you:

- Do not eat anything.
- Do not drink liquids (this includes any type of liquid: water, coffee, alcohol, etc.).
- Do not brush your teeth.
- Do not smoke or use tobacco products.
- Do not use mouthwash.
- Do not engage in exercise or similar physical activity.

Please wait to brush your teeth until after you have collected BOTH of your morning samples. However, even if you do any of the things on the list, it is important to go ahead and collect your saliva sample at the normal time. Just be sure to note in the diary what you did before collecting the sample.

Please complete the saliva diary after each sample collection. The diary is a sheet of paper included in each box. The diary will ask you to write down the date and time of each sample collection and respond to additional questions related to the behaviors listed above and sample storage. PLEASE REMEMBER TO RETURN THE DIARY ALONG WITH YOUR SALIVA SAMPLES WHEN YOU SHIP THEM TO US. You may place the diary in the envelope provided and put it on the top of the insulated container before you tape the box closed (see shipping instructions).

How to Use the Oral Swab

1. Remove the Ziploc bags from the silver pouch inside the insulated container in your “Start of Study” kit. Inside, there is a Ziploc bag with tubes for each day: Day 1, Day 2, and Day 3 of collection. On the day of collection, remove the tubes from the Ziploc bag for that day and put them in a safe place. Do not open the tubes until you are ready to collect a saliva sample. There are three tubes for each day, one for each sample that you will collect. Each tube is labeled with the day (Day 1, Day 2, Day 3) and time of collection (wake up, 30 min. after wake up, and bedtime).
2. Remove the cap and take the oral swab from the correct tube.
3. Place the oral swab under your tongue and keep it in place for 1-2 minutes or until it is completely soaked with your saliva. Try not to move the swab around in your mouth.
4. After the oral swab is soaked with saliva, put it back into the upper part of the same tube that you took it from (as shown in the picture below). Replace the cap, snap it securely onto the tube and put the tube back into the bag that you got it from.
5. Store the Ziploc bags with completed samples in the freezer immediately after collecting.

6. Once all nine samples have been completed, please use the attached packaging instructions in the kit to pack the insulated container and the shipping box ON THE DAY THAT YOU WILL SHIP IT.

7. The box should be shipped back to us on the next Monday, Tuesday, or Wednesday (that is not a holiday) after you have collected all of your samples. You may take the shipper to a local FedEx store to ship or you can call FedEx at 1-800-GoFedEx (1.800.463.3339) to have the package picked up at your home. Please note that FedEx requires someone to be home when a package is picked up.

**If at any point, you have questions regarding collecting or shipping your samples, please call Ms. Gulya Kourman at (336) 716-2581.**
Saliva Collection Diary

Day 1

Date _____/_____/

Time 1 (When you woke up) _____:_____ am

1. Did you eat 30 minutes before collecting your saliva? YES NO
2. Did you drink any liquids 30 minutes before collecting your saliva? YES NO
3. Did you drink alcohol 30 minutes before collecting your saliva? YES NO
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva? YES NO
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva? YES NO
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva? YES NO
7. Was the sample taken on time? YES NO

Time 2 (30 minutes after you woke up) _____:_____ am

1. Did you eat 30 minutes before collecting your saliva? YES NO
2. Did you drink any liquids 30 minutes before collecting your saliva? YES NO
3. Did you drink alcohol 30 minutes before collecting your saliva? YES NO
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva? YES NO
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva? YES NO
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva? YES NO
7. Was the sample taken on time? YES NO

Time 3 (When you went to bed) _____:_____ pm

1. Did you eat 30 minutes before collecting your saliva? YES NO
2. Did you drink any liquids 30 minutes before collecting your saliva? YES NO
3. Did you drink alcohol 30 minutes before collecting your saliva? YES NO
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva? YES NO
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva? YES NO
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva? YES NO
7. Was the sample taken on time? YES NO

Were all of your samples from this day stored in the freezer? YES NO
Saliva Collection Diary

Day 2

Date _____/_____/_____

Time 1 (When you woke up) _____:______ am

1. Did you eat 30 minutes before collecting your saliva? YES NO
2. Did you drink any liquids 30 minutes before collecting your saliva? YES NO
3. Did you drink alcohol 30 minutes before collecting your saliva? YES NO
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva? YES NO
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva? YES NO
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva? YES NO
7. Was the sample taken on time? YES NO

Time 2 (30 minutes after you woke up) _____:______ am

1. Did you eat 30 minutes before collecting your saliva? YES NO
2. Did you drink any liquids 30 minutes before collecting your saliva? YES NO
3. Did you drink alcohol 30 minutes before collecting your saliva? YES NO
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva? YES NO
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva? YES NO
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva? YES NO
7. Was the sample taken on time? YES NO

Time 3 (When you went to bed) _____:______ pm

1. Did you eat 30 minutes before collecting your saliva? YES NO
2. Did you drink any liquids 30 minutes before collecting your saliva? YES NO
3. Did you drink alcohol 30 minutes before collecting your saliva? YES NO
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva? YES NO
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva? YES NO
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva? YES NO
7. Was the sample taken on time? YES NO

Were all of your samples from this day stored in the freezer? YES NO
Saliva Collection Diary

Day 3  
Date  ____/____/_____

Time 1  
(When you woke up)  ____:____ am

1. Did you eat 30 minutes before collecting your saliva?  
2. Did you drink any liquids 30 minutes before collecting your saliva?  
3. Did you drink alcohol 30 minutes before collecting your saliva?  
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva?  
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva?  
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva?  
7. Was the sample taken on time?  

Time 2  
(30 minutes after you woke up)  ____:____ am

1. Did you eat 30 minutes before collecting your saliva?  
2. Did you drink any liquids 30 minutes before collecting your saliva?  
3. Did you drink alcohol 30 minutes before collecting your saliva?  
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva?  
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva?  
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva?  
7. Was the sample taken on time?  

Time 3  
(When you went to bed)  ____:____ pm

1. Did you eat 30 minutes before collecting your saliva?  
2. Did you drink any liquids 30 minutes before collecting your saliva?  
3. Did you drink alcohol 30 minutes before collecting your saliva?  
4. Did you brush your teeth or use mouth wash 30 minutes before collecting your saliva?  
5. Did you smoke or use tobacco products 30 minutes before collecting your saliva?  
6. Did you do any physical activity or exercise 30 minutes before collecting your saliva?  
7. Was the sample taken on time?  

Were all of your samples from this day stored in the freezer?  

YES  NO
Sample Kit Shipping Instructions

1. Remove the three Ziploc collection bags containing the nine collected saliva samples from the freezer.
2. Place the three bags containing the tubes into the large Ziploc bag with the absorbent paper pad provided. Close the Ziploc bag and squeeze out as much of the air as possible.
3. Place the large Ziploc bag between the halves of the frozen gel pack like you are making a sandwich.
4. Insert the frozen gel pack “sandwich” into the insulated silver colored Ziploc bag and seal the Ziploc.
5. Place the packed silver colored foil Ziploc bag into the Styrofoam box so that it stands on its edge across the opposite corners of the box.
6. Place a frozen ice brick on each side of the silver colored foil Ziploc.
7. Replace the lid on the Styrofoam box.
8. Place the “Saliva Collection Diary” in its envelope and place the envelope on top of the Styrofoam box.
9. Put the saved bubble wrap on top of the envelope and seal the cardboard box with packaging tape. Be careful NOT to place tape over the FedEx shipping label already attached to the box.
10. Ship the package to the address on the FedEx label on the next Monday, Tuesday, or Wednesday that is not a holiday after you have collected the last sample. You may take the package to a local FedEx store to ship or you can call FedEx at 1-800-GoFedEx (1.800.463.3339) to have the package picked up at your home. Please note that FedEx requires someone to be home when a package is picked up.
11. Contact your local clinic or you may contact us using the phone number below if you have any questions

Ms. Gulya Kourman
RELAX Cortisol Study
Wake Forest Biotech Place
575 Patterson Avenue, Suite 250
Winston-Salem, N.C. 27101
Phone: (336) 716-2581
Site Coordinator Instructions for Cortisol Collection

When the protocol is approved at your site, a shipment of cortisol kits will automatically be sent. For additional cortisol kits, send an e-mail to Gulya Kourman at kkourman@wakehealth.edu.

Materials for saliva collection will be distributed to participants in person at the baseline visit when they receive their device. For consistency, one complete cortisol kit must be used for each participant. Cortisol kits should be opened by the participant at home and not by site personnel.

Each cortisol kit will consist of one box which contains 2 inner shipping boxes, one labeled “Start of Study” (baseline) and one labeled “End of Study” (12-week follow-up). Each shipping box will contain the following:

- Instructions for saliva collection for the participant
- Saliva diaries
- Pre-addressed FedEx shipping label
- 3 Ziploc bags (Day 1, Day 2, and Day 3). Each has three tubes containing an oral swab. The tubes are labeled wake up, 30 minutes after wake up, and bedtime.
- A larger Ziploc bag with an adsorbent pad in which the three sample bags will be enclosed.

Your site has been provided with a “Demo Kit.” It is very important that you: (1) familiarize yourself with the Participant Instructions and Demo Kit prior to recruiting participants; and (2) contact the lab listed below if you have any questions. Site coordinators should review the Demo Kit and instructions for collecting, storing, packaging, and shipping samples with each participant.

Participants should be instructed to refrain from eating, drinking, smoking or using tobacco, brushing their teeth, using mouthwash, or engaging in exercise or similar physical activity for 30 minutes prior to saliva collection. They will be provided with diaries to record compliance with these behaviors as well as the date and time when their saliva samples were collected.

Participants will collect saliva samples three times each day (wake up, 30 minutes after wake up, and bedtime) for three consecutive days following baseline and three consecutive days following the 12-week follow-up. Saliva samples are collected by placing an oral swab under the tongue for approximately 1-2 minutes or until saturated with saliva. The oral swab will subsequently be stored in a pre-labeled plastic tube, placed in a Ziploc bag, and immediately placed in the freezer.

Samples are to be shipped twice, once after the first three days of samples have been collected at baseline (Start of Study) and again after the last three days of samples have been collected at the 12-week follow-up (End of Study). Participants will mail the compliance diaries with their saliva samples using the pre-addressed FedEx shipping label to Dr. Mark Lively’s laboratory (Wake Forest NCORP Research Base Biospecimen Lab) at the following address:

RELAX Cortisol Study  
c/o Ms. Gulya Kourman/Lively Lab  
Department of Biochemistry  
Wake Forest BioTech Place  
575 Patterson Avenue, Suite 240-250  
Winston-Salem, N.C. 27101  
Phone: (336) 716-2581
Site Coordinator Instructions for Cortisol Collection

Sample kits are to be shipped by FedEx Priority Overnight Delivery only on a Monday, Tuesday, or Wednesday (except holidays). A completed, pre-paid shipping label is attached to each shipping box. Participants will NOT have to pay for shipping. The shipping label is complete with our address as the return address. Participants may take samples to a FedEx location for shipment or they may call 1-800-GoFedEx (1.800.463.3339) to schedule a pick-up at their home. FedEx requires someone to be home when the package is picked up. Please encourage participants to call the site coordinator if any problems occur.