At-Home Study Kit Instructions

Welcome! Thank you for participating in the Circadia research study!

We are a clinical research study located at Massachusetts General Hospital dedicated to better understanding the genetic influences of atypical circadian rhythms. Our study is self-directed and completely home-based, making study participation far reaching, flexible, and focused on your schedule.

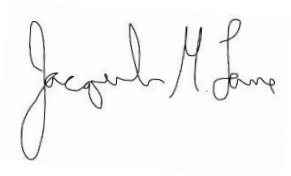
The Circadia research team, led by Dr. Jacqueline Lane, is interested in better understanding the genetics of circadian rhythms, especially atypical circadian rhythms. Circadian rhythms are an integral part of our functioning, regulating and syncing our human behavior and physiology with the external environment and the 24-hour day. While research has been able to identify core components of our molecular circadian clock, much is still unknown about the genetics and physiological mechanisms driving our circadian rhythms.

Working together, we believe that we will be able to make the genetic pathways related to circadian rhythms clearer, identify the genetic components directly influencing atypical circadian rhythms, and through this be able to better help inform the diagnosis, treatment, and therapies for all who are living with an atypical circadian rhythm.

If you have this letter and instruction packet in hand that means you have successfully received our at-home study kit. In this packet you will find details about the study and your study kit, including:

Additional instructions can be found on the participant portal at circadiastudy.org. As always, please reach out to us with any questions, concerns, or thoughts.

Best Regards,



Jacqueline Lane, PhD
Principal Investigator of the Circadia Study

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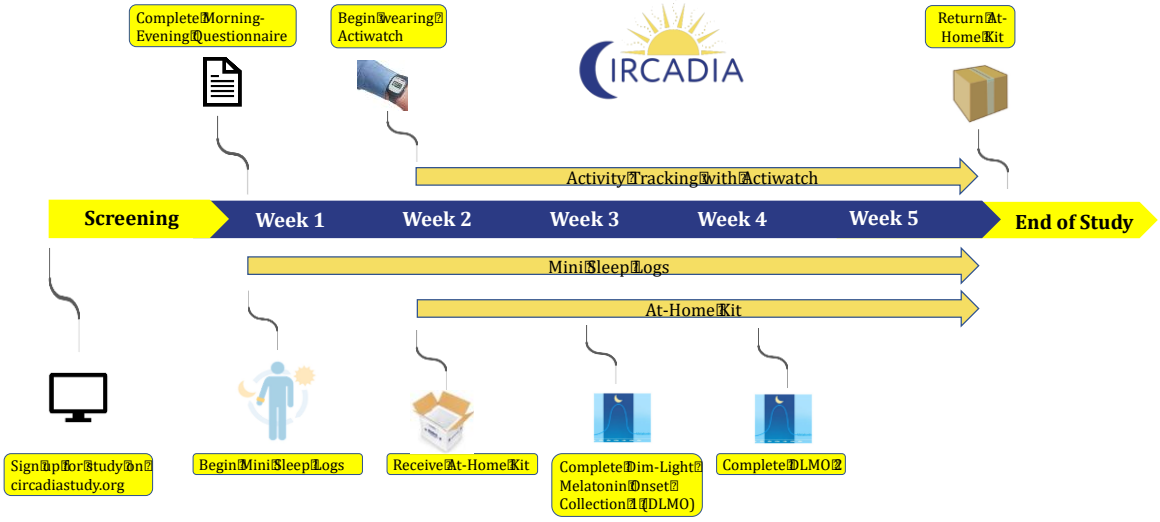
Overview of the Circadia Study

Circadia Study Timeline



The Circadia study is a 4-6 week study.

During the study you will complete:

- One Morningness- Eveningness Questionnaire
- A Mini Sleep Log each day for 4 weeks
- Two Dim-Light Melatonin Onset Collections (DLMOs)

See the study portal CircadiaStudy.org to complete the questionnaire and sleep logs.

This at-home kit is used for:

- Two dim-light melatonin collections (DLMOs)
- Activity tracking with the Actiwatch

Collection Goals for the Circadia At-Home Study Kit

This kit was created with you in mind, to help you easily and successfully provide your study collections. These include the following: activity and behavior monitoring and a measure of your melatonin levels provided through saliva samples collected hourly in dim light conditions. Below we explain the study Collection Goals:

Goal 1) Complete Study Monitoring and Logging

An important aspect of the Circadia study is better understanding the attributes of your daily activity, light exposure, and sleep and wake time. We will measure your activity and light exposure through a watch-like wearable called an **Actiwatch.** We will assess your sleep behavior and mood using surveys and applications.

Goal 2) Provide hourly melatonin saliva samples in a dim light environment

Melatonin is a hormone that helps regulate our sleep-wake cycle. You will schedule the two dates for the melatonin sample collections, set one week apart from one another, based on your schedule. We have provided scheduling, preparation, and collection instructions on **pages 8-14**. To achieve this goal, you will use the following:


Bottle with time stamp lid and cotton collection swabs


Temperature monitor

Misc. Items: UVEX Blue light-blocking glasses, toothbrush, tape, icepack, insulated pouch.


Night lights and light meter


Collection Tubes

Study Checklists and Guides

Study Kit Components Checklist

Electronics

☐  Actiwatch in a labeled envelope (1)

☐  Light Meter in a labeled envelope (1)

☐  Battery Operated Tea Lights (18)

☐  iButton (1 in Ziploc and 1 pre-taped in silver envelope) (2)

Collection Tools

☐ Clear bag labeled for DLMO collection 1 containing the following:

- Bottle with time stamp lid
- 9 cotton collection swabs inside of the bottle with time stamp lid
- 9 empty collection tubes
- 12 labels, 9 for sample collection and 3 extra
- 1 toothbrush
- 1 Sharpie

☐ Clear bag labeled for DLMO collection 2 containing the following:

- Capped bottle containing 9 cotton collection swabs
- 9 empty collection tubes
- 12 labels, 9 for sample collection and 3 extra
- 1 toothbrush

Other Study Materials

☐ Aluminum foil box

☐ 6 Black Trash Bags

☐ UVEX Blue light-blocking glasses

☐ Painters Tape

☐ 2 freezer packs, each in a resealable plastic pouch

☐ Clear bag with the following:

- Roll of medical tape
- 2 Tylenol single-use packs
- 2 extra collection tubes with cotton swabs

Shipping Materials

☐ Silver envelope labeled for DLMO Collection 1

☐ Silver envelope labeled for DLMO Collection 2 with iButton taped inside

☐ Return shipping label

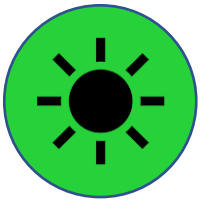
☐ Return shipping pouch

☐ Return shipping tape

☐ Return shipping box (same as kit box)

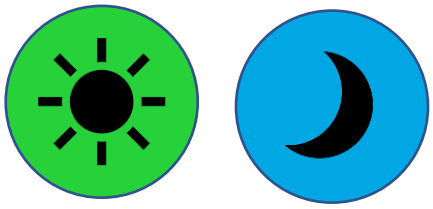
Study Component Guide

To help you navigate through study components included in the At-Home kit, the following labels indicate what materials are used for the two Dim Light Melatonin Onset (DLMO) Collections.



A green circle with a sun symbol indicates DLMO Collection 1 components. You will use these materials for DLMO Collection 1.

A blue circle with a moon indicates DLMO Collection 2 components. You will use these materials for DLMO Collection 2.



Any material with both a green sun circle and a blue moon circle indicates you will use this item for both DLMO Collection 1 and DLMO Collection 2.

Goal 1: Complete Study Monitoring using Actiwatch and Mini Sleep Log

Actiwatch Instructions

- Wear the Actiwatch for the duration of the at-home protocol. This will be for 24 hours a day for about 4 weeks.
- The Actiwatch may be worn while showering or washing your hands. You may take the Actiwatch off if you prefer to not get it wet. If you remove it, please put it on immediately after your activity.
- Prior to going to sleep, press and hold the long button on the right side of the Actiwatch until you hear a beep. This will mark the start of your bedtime.
- Immediately after waking up, press and hold the long button on the right side of the Actiwatch until you hear a beep. This will mark your wake time.
- If the Actiwatch at any point causes skin sensitivity, use the provided medical tape to wear underneath the band. If the sensitivity persists, you may remove the Actiwatch. Contact the study team if this occurs.

Using the Actiwatch

To use the Actiwatch, unhook the clasp and place the band on your **non-dominant hand**.

For example, if you write with your right hand, you will wear this on your left hand for the duration of the study.

Adjust the strap then clasp it, ensuring a snug but comfortable fit. Ensure the face of the Actiwatch is not covered by any clothing.

Mini Sleep Log

Complete the Mini Sleep Log each day on the Patient Portal at circadiastudy.org

Daily Study Checklist

The following checklist is for daily use for the duration of the Circadia Study. Use the calendar to keep track of completed days.

☐ **Complete Mini Sleep Log**

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Week 1	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log
Week 2	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log
Week 3	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log
Week 4	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log
Week 5	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log
Week 6	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log	☐ Sleep Log

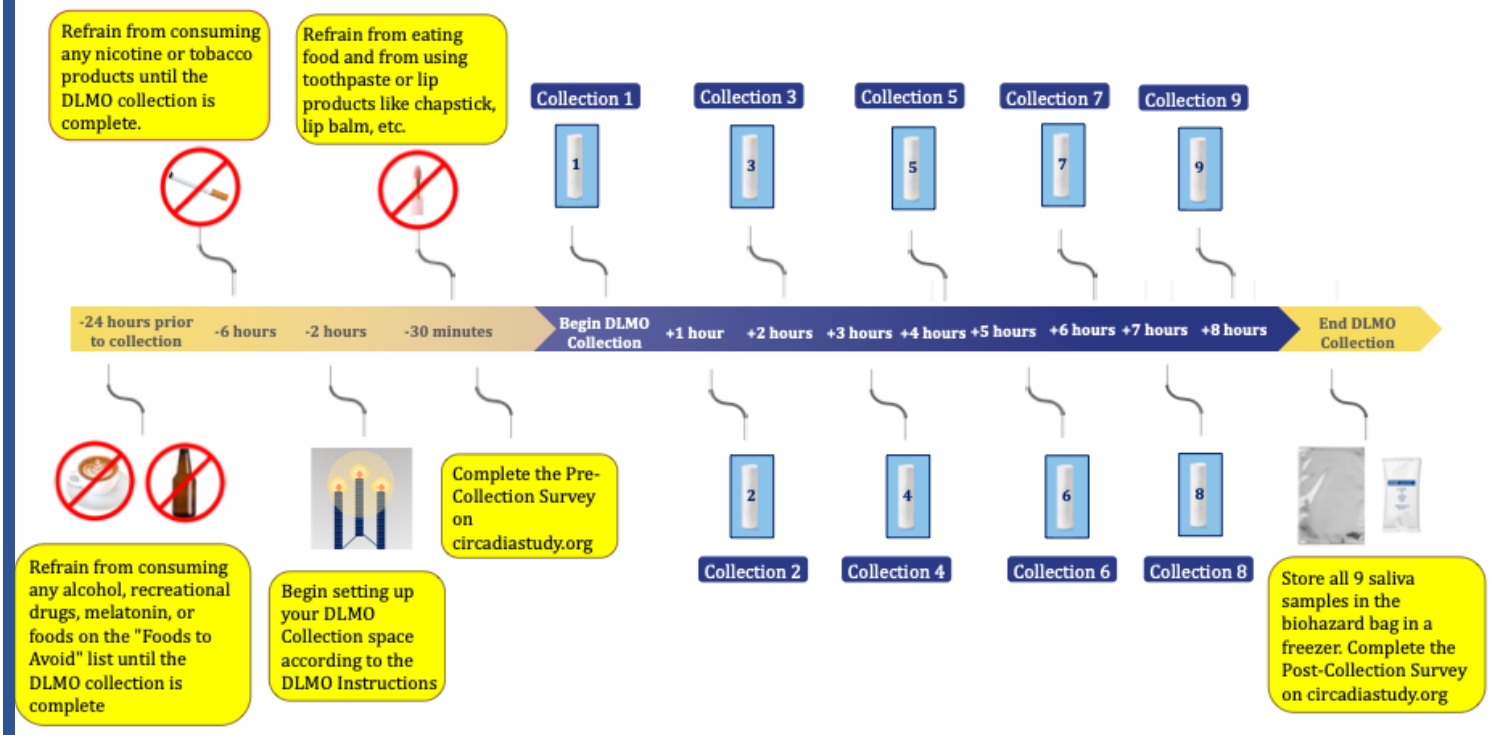
Goal 2: Provide Hourly Melatonin Saliva Samples in a Dim Light Environment

Dim Light Melatonin Onset (DLMO)
Saliva Sample Collection

Overview: The Dim Light Melatonin Saliva Sample Collection kit is designed to collect saliva samples in one-hour intervals for 8 hours under dim light conditions and overlapping with your bedtime. The samples will be analyzed for melatonin concentration, which provide us insight on your biological bedtime. Melatonin is a hormone that regulates your sleep-wake cycle. **Melatonin levels are affected by light**. For this sample collection, it is extremely important to follow instructions carefully to ensure samples are not affected by light levels. We will be tracking the times of saliva collection so please make every effort to follow scheduled sample times and to open and close the bottle with time stamp lid containing the sample collection cotton swabs fully after each collection.

Protocol Duration: ~8 hours
Attention: Icepacks must be **frozen** prior to start of sample collection

DLMO Collection Timeline



3 Steps for DLMO Sample Collection Success

1. Your food, drink, and medication: Monitor your **food, drink, and medication** consumption for 24 hours prior to, and during the study.

2. Your room: Keep your room dim and relaxed during the study.

3. Your saliva samples: Keep samples free of food, collect them on time, and store them cold.

The following pages [16-22] describe how to complete these steps.

Step 1. Monitor your food, drink, and medications.

24 Hours
Prior to Collection

No alcohol, caffeine, or recreational drug consumption

Day of Collection

No alcohol, caffeine, or recreational drug consumption.

Avoid the following:
Food: Turkey, bananas, citric acid, foods containing Red 40
(see Foods to Avoid)

6 Hours
Prior to Collection

No alcohol, caffeine, or recreational drug consumption.

No nicotine or tobacco products including but not limited to cigarettes, vaping, chewing tobacco, pipes, cigars.

Do **NOT** consume the following:

Drinks: Coffee, energy drinks, caffeinated tea, caffeinated soda,

Food: Turkey, bananas, citric acid, foods containing red 40, chocolate
(see Food Items to Avoid)

Immediately before and during collection

No toothpaste when brushing teeth.

No lip products such as chap stick, lip balm, or lip stick.

Melatonin Sample Collection: Food Items to Avoid

The following is a list of food items to avoid when preparing for the melatonin sample collection. Melatonin levels can be affected by food; therefore, it is very important to avoid any food or drink that may influence your melatonin levels. The following list is for guidance but is not exhaustive. Contact the study team if you are unsure whether a snack or drink is compliant with the study protocol.

Food Containing Red 40

Cereal

Fruity Pebbles, Fruity Cheerios, Trix, Cap'n Crunch Berry Flavor

Food

Pickles, some cherry pie fillings, some barbecue sauces

Drinks

Powerade Orange, Crush, Sunny Delight Orange Strawberry

Candy

M&Ms, Reese's Pieces, Strawberry Twizzlers, Skittles, Peeps, Candy Corn, Jellybeans

Food Containing Citric Acid

Drinks

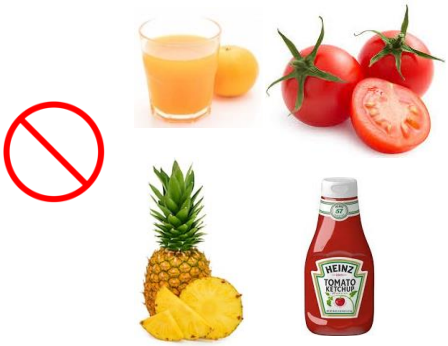
Orange Juice, Grapefruit Juice

Fruits

Lemons, limes, oranges, grapefruits, tangerines, pomelos, pineapple, strawberries, raspberries, cranberries, cherries, tomatoes

Other

Ketchup



Food and Drink Containing Caffeine

Drinks

Coffee, Soda, Energy Drinks, Tea (Black, Green, White)

Food

Chocolate, Chocolate Flavored Cereal, Chocolate Pudding, Chocolate Cake, Ice Cream (Chocolate, Coffee flavors)

Other

Excedrin Pain Reliever

Melatonin Sample Collection: Acceptable Food Options

The following is a list of acceptable food options in case you are hungry and choose to eat during the sample collection. Melatonin levels can be affected by food; therefore, it is very important to choose snacks and drinks that are compliant with the study protocol. The following list is for guidance but is not exhaustive. Contact the study team if you are unsure whether a snack or drink is compliant with the study protocol.

Drinks

- Caffeine-free herbal teas
  - *Mint tea*
  - *Chamomile*
- Caffeine-free sodas
  - *Root-beer*
- Milk, Plain or Chocolate
  - *Cow Milk*
  - *Almond Milk*
  - *Soy Milk*
  - *Coconut Milk*
- Flavored Seltzer or Sparkling Water

Snacks

- Cup Snacks
  - *Apple sauce*
  - *Vanilla pudding*
- Crackers
  - *Triscuits*
  - *Cheez-Its*
  - *Goldfish Crackers*
  - *Rice Thins (gluten-free)*
  - *Stauffer's Animal Crackers*
  - *Graham Crackers*
- Granola bars
  - *Kashi Granola Bars (Honey Almond Flavor)*
  - *Nature Valley Brand (Oats n Honey, Cinnamon, Peanut Butter)*
- String Cheese
- Light butter popcorn

Fruits

- Apples
- Pears
- Watermelon
- Raspberries
- Blueberries
- Cantaloupe

Step 2. Keep your room dim and relaxed

Part 1: Preparing your Environment

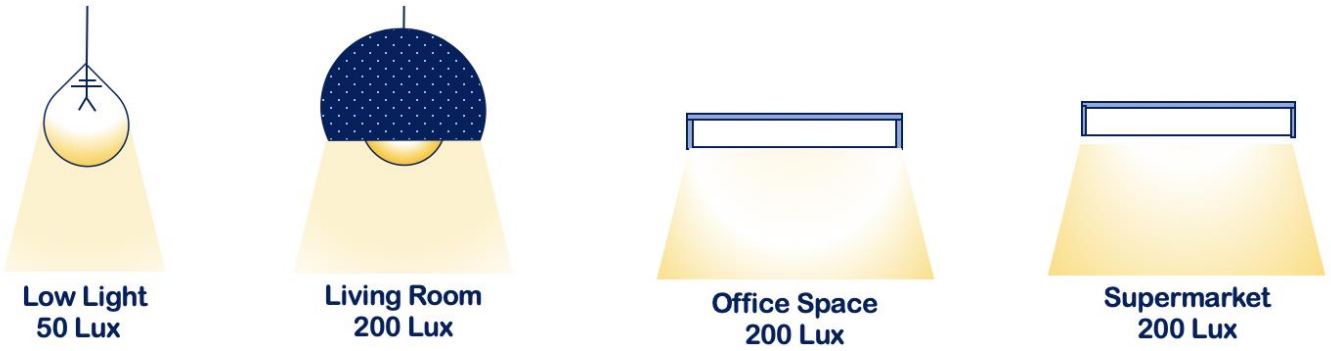
This protocol needs to be conducted in an area of rest, where you won't be disturbed or exposed to bright light for the entirety of the collection period, about 8 hours. For this protocol a dim light environment below 10 lux must be maintained. A light meter and tea lights have been provided to facilitate maintaining dim light conditions. Blue light blocking glasses have been provided to minimize exposure to blue light. The following instructions will help you prepare your area of rest to ensure proper dim light conditions are maintained for the duration of the protocol:

1. Using the provided tape, tape down light switches in your collection space, nearest restroom, and any other areas where light may be needed during the protocol. You may need to tape the provided aluminum foil or black trash bags over windows to block light.
2. Place up to 16 of the provided battery-operated tea lights in your collection space and two battery-operated tea lights in your bathroom. This will ensure dim light is maintained if the bathroom is needed during the protocol.
3. The provided light meter is handheld. When you are ready to begin sample collections and you have set your environment to dim light conditions, use the light meter to measure your light exposure. The meter should read below 10 lux. Please record this value in your diary.
4. When dim light conditions under 10 lux have been achieved, you are ready to begin the protocol.
5. You may only use electronics such as a TV or cellphone if they have been dimmed to the lowest setting. If using either of these devices, change their settings to the dimmest light setting before beginning the sample collection. You may use a laptop provided it is on the dimmest setting, with night mode activated if possible, and the laptop is AT LEAST 2 feet (24 inches) away from your face. Electronic tablets (for example iPads, Kindles) are not allowed. You must take these precautions the whole 8 hours.

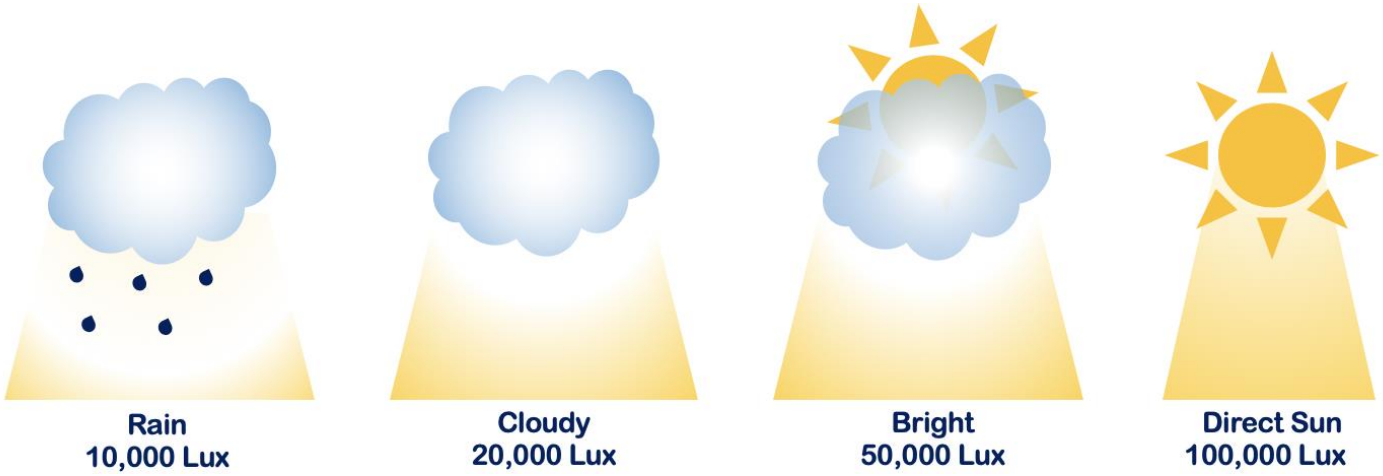
Melatonin Sample Collection: Guidance on Light Intensity

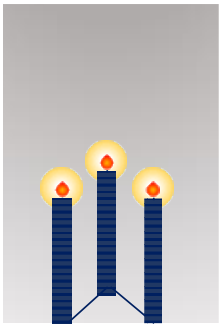
The diagram below depicts different light settings and their approximate lux. Lux is a measure of light intensity, equal to one lumen per square meter. That means that in a darkened room, one lux is approximately equal to the light emitted from one lit candle. Melatonin levels are diminished by higher lux levels; therefore, it is important for the melatonin sample collection to collect samples in dim light conditions.

Internal Light Lux Measures



External Light Lux Measures





For the melatonin sample collection, samples must be collected in dim light conditions.

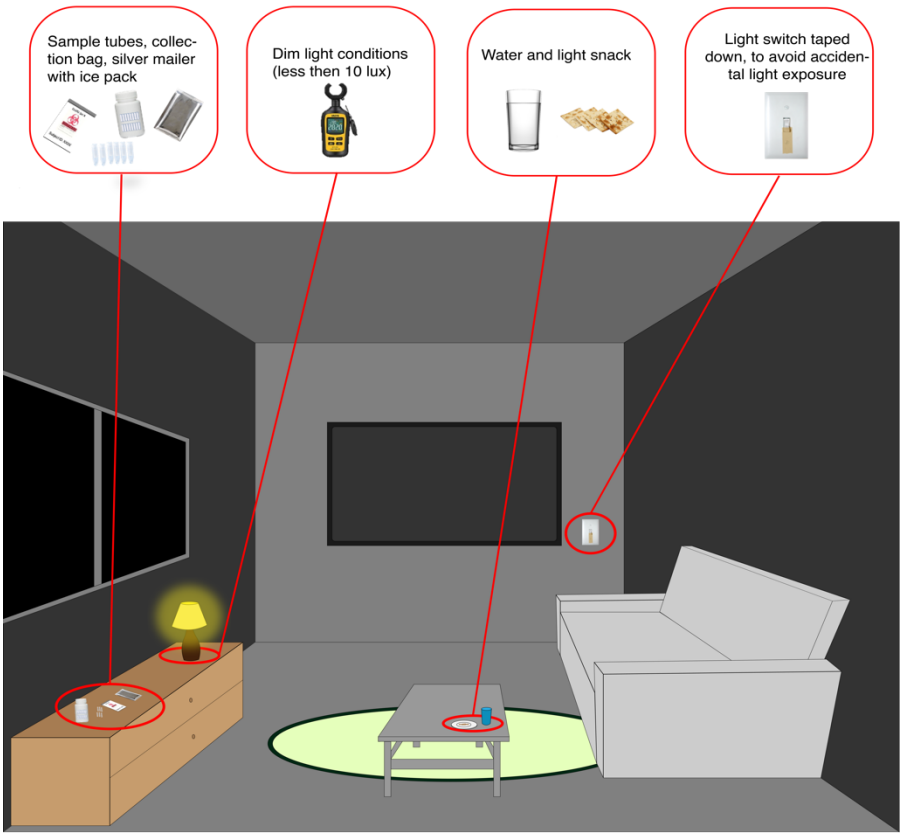
Dim light conditions are defined as a collection space with light measuring below 10 lux.

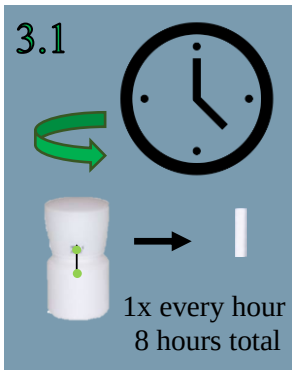
Part 2: Preparing for Collection

This protocol requires that you maintain a resting position for the duration of the protocol. The following instructions will prepare you to ensure resting conditions are maintained for the duration of the protocol:

1. You may place water and a snack near you in case you are thirsty or hungry during the protocol. Please see the provided guidance sheets for a list of study protocol appropriate food. *You may drink water or eat immediately* **AFTER** *a saliva sample collection, giving at least 30 minutes prior to your next collection and using a wet toothbrush with NO toothpaste to clean mouth and teeth.*
2. Two bags of collection tubes have been provided; each bag contains 10 tubes. Take one bag of tubes and place the bag near you where tubes can be accessed easily.
3. You will label tubes as you collect samples during the protocol. A set of labels is included in each sample collection bag.
4. Collected saliva samples will need to remain cold during the protocol. Ice packs and silver envelopes were provided with the collection kit. Place one frozen ice pack into the silver envelope and place it near you, where it can be accessed easily.
5. Note for DLMO 2 ensure that you are using the same cap that you did for DLMO 1.

Example Room Set Up





Step 3. Collect and store your saliva samples.

1. Open the bottle with time stamp lid at the scheduled sample collection time and remove **one** cotton collection swab then replace the cap and twist it closed until the two green circles align. The cap will automatically track the time opened and closed.
2. Place the cotton collection swab in your mouth, letting it sit under your tongue until it becomes saturated with saliva, approximately 3-4 minutes.
3. Once the cotton is fully saturated, remove it from your mouth and place it into a collection tube. Using the provided marker, mark the tube label with the sample number, for example 1 for Sample 1.
4. Securely fasten the cap onto the tube.
5. Place the completed sample into the biohazard bag, then place and keep the bag inside of the silver pouch with the ice pack.
6. Repeat steps 1-6 every hour on the hour until all nine samples are collected. At the end of the protocol, you should have nine samples labeled “Sample 1; Sample 2; Sample 3…”.
7. Place completed samples into the biohazard bag. Place that bag into the silver pouch containing a frozen icepack. Continue adding collected samples until all nine samples are completed. Place the silver pouch containing the icepack and biohazard bag with samples into your freezer.

- Store all saliva samples in the silver pouch with frozen icepack
- Once ALL 9 samples are complete, place entire silver pouch with icepack and samples in your **FREEZER**
- Keep samples in freezer until ready to return samples
- Mark each collection complete in the portal

Study Completion

Study Completion: Returning Samples and Electronics

The following instructions are provided to ensure collected samples are shipped appropriately. Icepacks should be fully frozen and remain in the silver envelopes with collected samples. Use the cardboard box and foam liner to return ship study samples and material. Please **ONLY** package samples on the same day as planned return shipping.

Melatonin Collection Samples

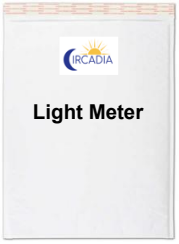
When ready to ship, place the silver envelopes for DLMO Collection 1 and DLMO Collection 2, containing frozen samples and frozen icepacks, into the foam box lining the carboard shipping box.

Shipping Temperature:
Frozen

Electronics

Place the Light Meter and Actiwatch in their original envelopes. Place these envelopes with electronics in the foam box lining the carboard shipping box.

Shipping Temperature:
Room Temperature



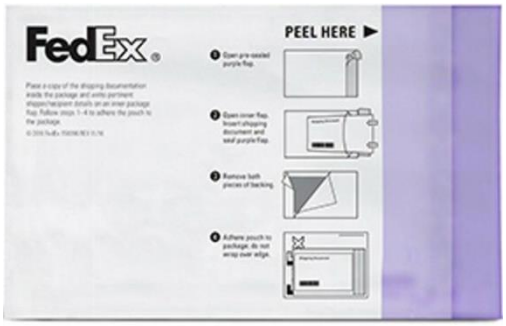
Refer to the Return Shipping: Item Checklist to ensure all study material is returned.

Study Completion: Shipping Samples and Electronics

The following instructions are provided to ensure collected samples are shipped appropriately. Please package samples on the same day as shipping.

1) Once samples and electronics are in their respective insulated mailers and inside the foam box, close the foam box using the foam lid. Then seal the cardboard box using the provided shipping tape.

2) Place the provided FedEx shipping slip into the FedEx pouch as shown below. Remove the sticker from the back of the FedEx pouch and adhere it to the top of the sealed box.



3) Bring the kit to your nearest FedEx center. (You can locate Fedex centers near you online at https://www.fedex.com/locate/.) If you need help finding your nearest FedEx center, please reach out to the study team and we will happily assist you. The FedEx center will scan the kit and mail it to our study team **at no cost to you.**

4) In the participant portal, navigate to the “Returning Samples and Electronics” tab and check off that you have returned the kit.

Return Shipping: Essential Items Checklist

Collected Samples:

☐  Silver envelope labeled DLMO Collection 1 containing 9 sample tubes from DLMO Collection 1 and an ice pack (iButton for DLMO 1 needs to be placed with the sample inside the silver envelope- treat it like any other sample).

☐  Silver envelope labeled DLMO Collection 2 containing 9 sample tubes from DLMO Collection 2 and an ice pack. Please also ensure that the iButton is still taped to the side of the silver envelope for DLMO 2.

Electronics:

☐  Actiwatch (1)

☐  Bottle with time stamp lid (1)

☐  Light Meter (1)

☐  Battery Operated Tea Lights (18)

☐  iButtons

Other:

☐  Blue Light-Blocking Glasses (1)

☐  Bottle with regular lid (1)

Thank you for participating in the Circadia Study!