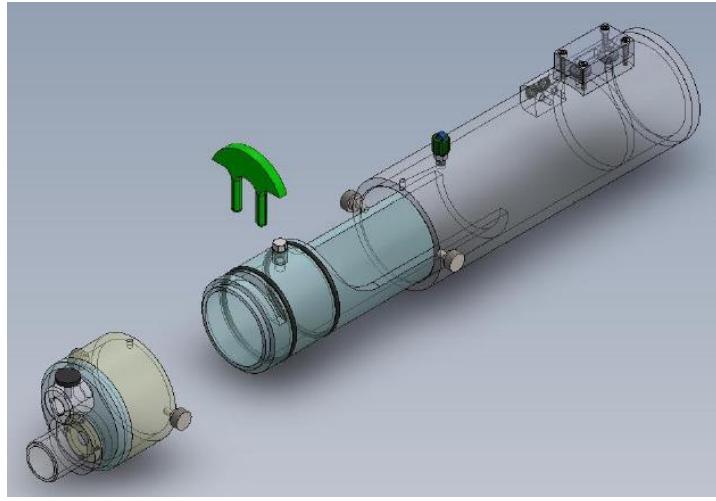


Allay Restraint Instructions



OVERVIEW

This document highlights typical steps needed to properly restrain and acclimate animals to DSI's Ally Restraint technology. It also provides feedback from the scientific community, recognizing individual animal handling skillsets differ slightly.



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REV02

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ALLAY RESTRAINT INSTRUCTIONS

OVERVIEW

The Allay restraint is a new and improved restraint system for use in rodents. This new design avoids issues typically seen with traditional rear-end or plunger type restraint devices.

The Allay restraint is used for the following applications:

- Head out chambers
 - Mouse: One chamber size available
 - Rat: Two chamber sizes available
- Inhalation tower nose-only restraints and plethysmographs
 - Mouse: One chamber size available
 - Rat: Four chamber sizes available
- Non-Invasive Airway Mechanics (NAM) chambers
 - Mouse: One chamber size available
 - Rat: One chamber size available

Note: Please see Body Weight section for more information on chamber sizes.

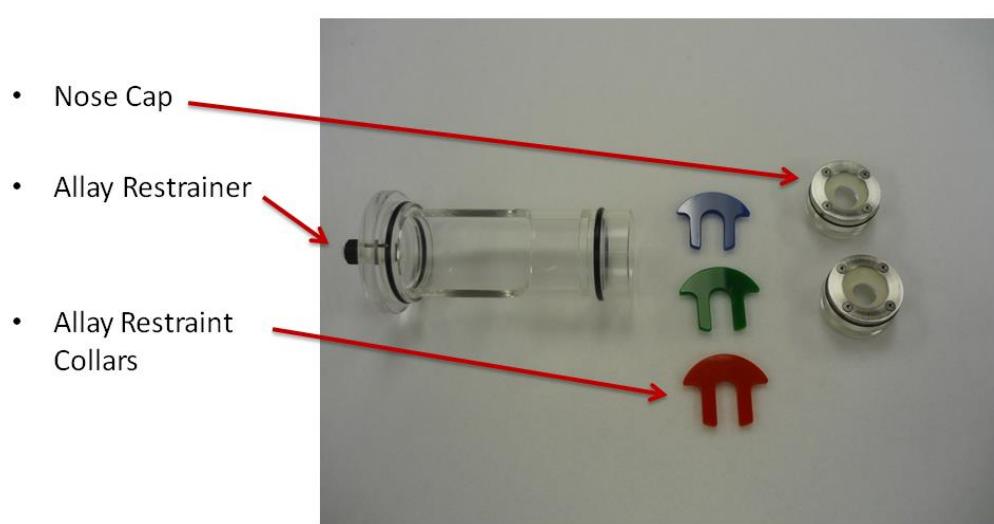
CHAMBER COMPONENTS AND SIZES

This section will depict the product components and estimated weight ranges for rodents.

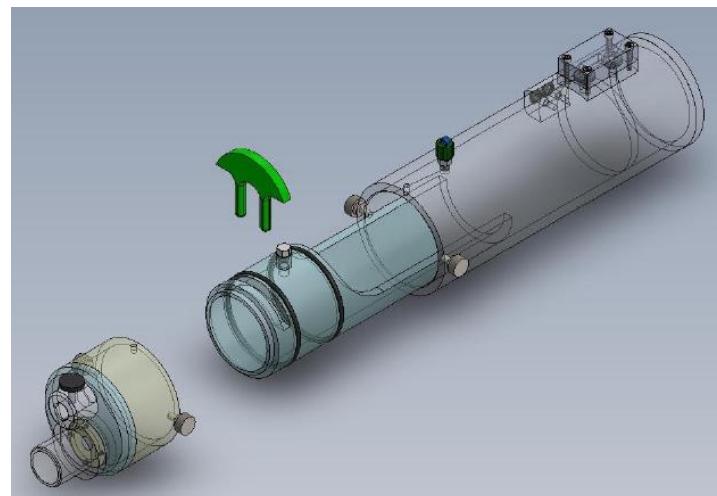
Note: Chambers components vary slightly based on species being used.

ALLAY RESTRAINT CHAMBER AND BODY WEIGHTS

- Restraint chambers and nose caps are used to properly restrain the animal. Sizes available will be dependent on the species being used
- The hole in the nose cap is not concentric and should be positioned with the hole down



Mouse nose-only restrainer



Rat nose-only restrainer with plethysmograph



Rat head out chamber

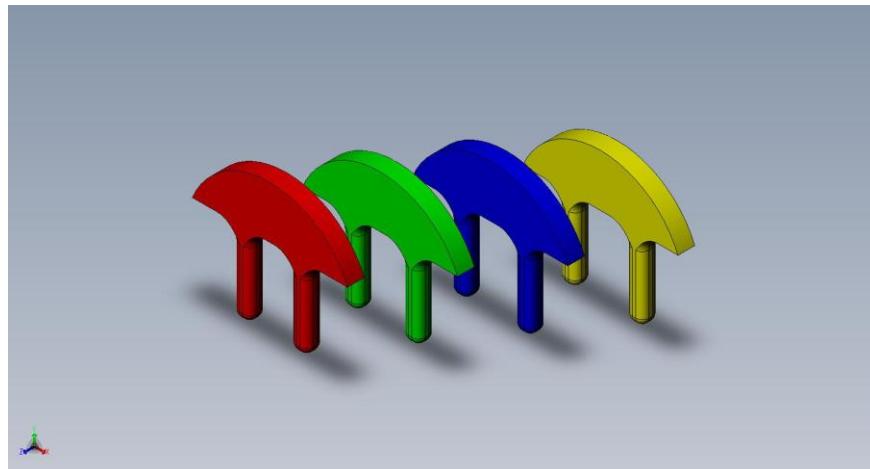
BODY WEIGHTS

- Body weights listed below are estimates. Care should be taken to ensure animal is properly restrained
- Rat chamber sizes (4) –**Note:** available sizes based on application
 - Standard: ~175-250 grams (Head-out, Nose-only, NAM)
 - Standard-Medium: ~250-325 grams (Nose-only)
 - Medium: ~325-400 grams (Nose-only)
 - Large: ~400-600 grams (Head-out, Nose-only)
- Mouse Chamber size (1)
 - Most commonly used mouse size is 20-25 grams (Head-out, Nose-only, NAM)

ALLAY RESTRAINT COLLARS AND NOSE CAPS

- The Allay restraint collars are used to properly restrain the animal. The appropriate size collar should fit snugly over the animal's neck, just behind the ears and in front of the shoulders. Nose caps are used to create a proper seal, allowing for inhalation/exposure and plethysmography studies
- Sizes available will be dependent on the species being used
- Rat:
 - Restraint collars come in 4 sizes: Red, Green, Blue, and Yellow
 - Red is the smallest while Yellow is the largest

- Nose caps come in one size and have one standard sized latex hole provided
- Mouse:
 - Restraint collars come in 4 sizes: Yellow, Red, Green, and Blue
 - Yellow is the smallest while Blue is the largest
 - Nose caps come in one size and can accommodate two different sized latex holes
 - Standard 3/8" (9.5mm) hole – included
 - Small 1/4" (12.7mm) hole – available; please contact local sales representative



Allay Restraint Collars

LOADING THE ANIMAL

This section depicts the various steps required to properly position and load the animal into an Allay Restraint chamber. Practice and confidence will allow a single investigator to easily perform this procedure.

Note: Animal position in the chamber may vary based on species and strain of animal being used.

CONSIDERATIONS

- If data collection is desired, it is suggested that hardware and software systems are ready for operation prior to placing the animal in the Allay restraint chamber
- The investigator should start with the largest restraint collar and work toward an appropriate size. Once comfortable with the process and the typical size of the animals used, choosing the appropriate collar will become easier
- O-rings can be lubricated using silicone grease to make it easier to insert/remove components. Place a small dab on your fingertip and run your finger around the entire O-ring. Do not leave a lot of greasy residue on the O-ring. A little grease goes a long way

SUGGESTED ANIMAL HANDLING PROCEDURE

1. Pick up the mouse by the base of the tail.
2. Load the animal into the rear of the Allay restraint and attempt to walk it along toward the open end



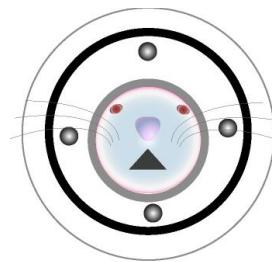
3. Once it reaches the other end of the restraint, apply more tension to the tail to hold the animal in place and ensure it does not travel too far. Use your judgment to decide when the animal is in a good position to drop the collar in place
4. Holding the animal and restraint in one hand, use your other hand to pick up one of the restraint collars
5. Carefully place the restraint collar over the animal's neck, behind the ears, and in front of the shoulder blades. Push the collar all the way down to secure the animal. Be careful not to catch any fur or skin when pushing down the collar
 - a. If the animal is able to back out, the collar is too big. Proceed to the next size collar

NOTE: Animals will inherently resist the process. Always observe the animal's behavior for any signs of significant stress and respond accordingly.

6. Once the appropriate sized collar has been properly placed, use an index finger to keep the Allay restraint collar in place and allow the animal to become acclimated to the restraint



7. Next, place the nose cap in your dominant hand, ensuring the non-concentric hole is facing down



Position of mouse (rats will not have eyes protruding beyond latex seal)

8. Slowly move the nose cap toward the animal. Allow the animal to adjust and find its way out through the hole, watching closely not to catch the animal's feet or whiskers
- A fresh latex seal is preferred so it will be more compliant

- b. After prolonged use, latex seals will harden and will need to be replaced
9. A properly placed nose cap is verified by ensuring the latex seal fits snugly over the nose and mouth and beyond the whiskers
- Allow the animal to become acclimated to this restraint

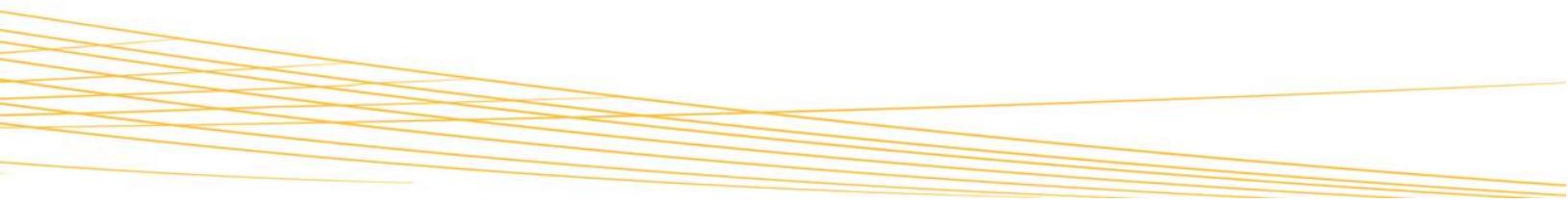


SUGGESTIONS FROM THE RESEARCH COMMUNITY

This section provides additional tips and suggestions to allow the investigator to become more comfortable with the Allay restraining process. These suggestions have been compiled from a number of Allay restraint users. They have been provided for your reference.

Acclimation is suggested prior to starting a study

- Investigators should consider acclimating animals to the restrainers prior to the start of a study.
Acclimating the animal allows for reproducible animal behavior and consistent data



Animal sedation is not required when using the Allay restrainer

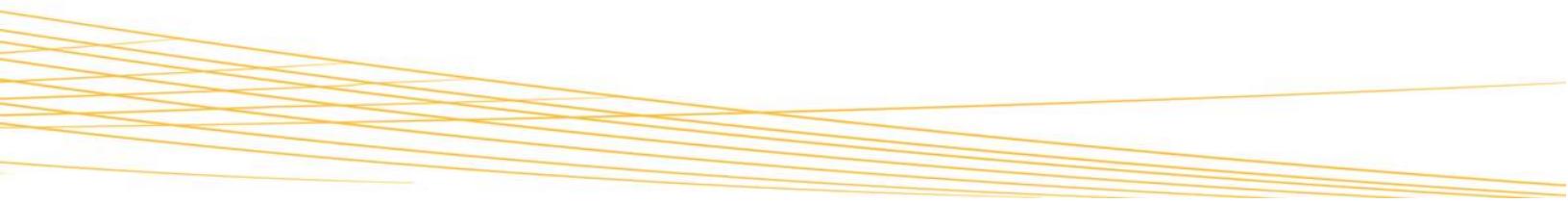
- Sedation may be useful for a new user trying the Allay restrainer for the first time
- Sedation should never be used while the animal is on a study

Location of loading an animal is important

- Do not hold the chamber in the air or over a ledge. The animal will feel more comfortable if they can see a flat surface beneath them
- If available, cover the front of the restrainer with a red, transparent material that still allows the investigator to properly place the Allay restraint collar. This may help further relax the animal

Removing the animal from the Allay restrainer

- It is recommended to remove the Allay restraint collar first and allow the animal to back out of the restrainer
- If the animal will not back out of the restrainer, carefully remove the nose cap
- Do not twist the nose cap when removing. Twisting the nose cap could result in serious injury or even death to the animal



TROUBLESHOOTING

TECHNICAL SUPPORT

Getting technical support from DSI will, we hope, be a quick and painless process, because we are strongly committed to helping you—our users—get the very best from our products. In other words, when you hit a road block, our aim is get your experiment, monitoring program, or whatever back up and running *as quickly as possible*. We are here to help!

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