



## Breeding NONcNZO10/LtJ (NON/NZ) and NON/ShiLtJ (NON/Shi) mice

Version: 1

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*(note that the following list should be linked to the appropriate location.)*

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### Summary:

- **NONcNZO10/LtJ (NON/NZ)** mice are the mice used for a Type 2 diabetes model. The mice are given 5K20 diet at weaning and the mice are usually exhibiting hyperglycemia by 10-11 weeks of age (see Figure 1) 90- 95 % of the mice will become diabetic at 20 weeks.
- **NON/ShiLtJ (NON/Shi)** mice are the mice used for a control to the diabetic mice. The mice are given 5K20 diet at weaning and should not become diabetic. But 5-10% of the mice will become diabetic at 20 weeks.
- Breeding, housing, and experimentation are performed in a pathogen-free environment

### Reagents and Materials:

*(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)*

Reagent/Material	Location	Quantity Required	Vendor	Stock Number
5K20 diet			Animals Specialties and Provisions, LLC	
2016 diet			Harlan Laboratories	

# Protocol:

## Breeding

**WARNING HAZARDOUS CONDITION WARNED AGAINST.** This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose the technician to the hazard.

1. Mice are bred at 6 to 7 weeks of age or later. Set up two females with one male.
2. Every 6 months new male and females breeders are purchased from JAX laboratories.
3. The mice are set up for 5 days. Typically the mice are set up on a Thursday and the male is separated on the Tuesday and the females remain together.
4. The Friday before the females are due to have pups, the cages are changed, and provided with fresh water and the food hopper is filled. Check to see which females are pregnant. Then place a "Do Not Disturb" sign on the cage.
5. For the week that the females start having pups, try not to move the cages in and out when trying to check for litters. Just try looking in the cages to see if you see any litters.
6. After the pups are born, wait a week and then remove the do not disturb sign. Fill water bottle and food hoppers if needed.
7. Cages that have two litters in same cage, check the sex of the pups at 2 to 2.5 weeks of age. Then divide the male pups evenly between both cages. If you have an odd number of males, place the extra male in one of the cages (he will be kept as a breeder). Then divide the females to even out both litters, giving both dams the same number of pups.
8. Wean the mice at 4 weeks of age (+/- 1 day from the date of birth). Wean the males in pairs. Any odd number males from litters will be kept as breeders. Females can be housed together, with up to 5 females per cage.

## Weaning

1. At weaning, the experimental NON/NZ & NON/Shi mice are placed on autoclaved for 20 min. at 120 C, 5K20 (Animals Specialties and Provisions, LLC ) diet. All breeder mice will be kept on 2016 (Harlan Laboratories diet).
2. Each experimental mouse will be ear tagged. The lowest ear tag number will be in the left ear of the one mouse and the highest ear tag number in the right ear of the other mouse. Ear tag the 1<sup>st</sup> mouse and then weigh the mouse. Place the mouse into the new cage. Then ear tag the 2<sup>nd</sup> mouse, then weigh and place into the new cage with 1<sup>st</sup> mouse ear tagged. Get a new cage and repeat ear tagging and weighing the next pair of mice.
3. Also wean the female mice at this time. The females are all placed on the 2916 diet and do not need weighed or blood glucose. The male breeders are also placed on the 2916 diet and do not need weighed or blood glucose.

## Weighing and Checking Blood Glucose Levels during the initial 19 wks of age

4. Mice are weighed every other week for the first four weeks on the 5K20 diet.

5. Then the mice are weighed and blood glucose levels are checked at 8 weeks old and then checked every other week after thereafter and measurements are taken up to 14 weeks on 5K20 Diet. At 6 weeks on 5K20 diet, a nestlet is placed into the experimental mice cages and the nesting sheets removed.
6. Mice should be weighed and blood glucose levels measured before 10:00am.
7. Mice are moved on Thursday's, usually at 19 weeks of age to CG740H in CAQ for experimentation
8. Mice that have blood glucose levels 250mg/dL or over are considered diabetic and glucose levels below 250mg/dL are controls. See Figure 1

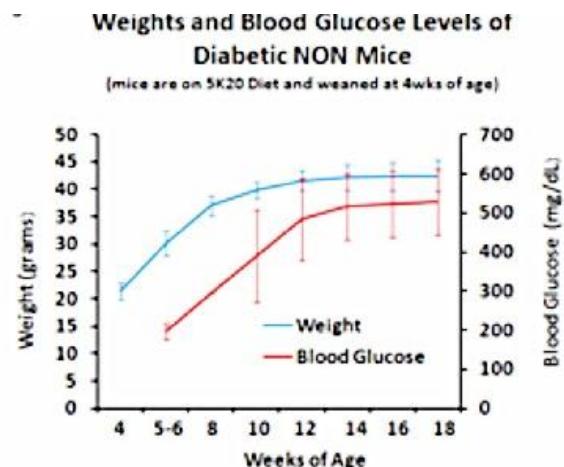


Figure 1 illustrates the relationship between the weight of the RCS10 mice and the onset of overt diabetes. Upon attaining a weight of 40 g, the mice all become diabetic which occurs between 10 and 14 weeks of age.

1. Perform step one.  
Explanation of what happens in this step.

*IMPORTANT: Important comments precede steps that, if performed incorrectly, will result in damage to equipment or to the samples.*

2. Perform step two.
  - a. Perform sub-step (a)  
Amplifying or more detailed information about the step.
  - b. Perform sub-step (b)

*NOTE: Additional tip that improves efficiency.*

3. Perform step three.

## **Reagent Preparation:** *(This area may have several different preparations with the table of contents below.)*

[Reagent 1](#) Autoclaved

[Reagent 2](#) Autoclaved

[Reagent 3](#)

### Reagent 1:

Reagents and Materials

Procedure

Checklist

### Reagent 2:

Reagents and Materials

Procedure

Checklist

### Reagent 3:

Reagents and Materials

Procedure

Checklist