





Protocol for Setting Up Timed Pregnant Rats and Mice

Rats and mice used for timed pregnant orders are bred in a similar fashion. Virgin female rats or mice are paired with proven breeder males just prior to the end of the daily light cycle. The following morning, each female is examined for the presence of an ejaculatory plug in the vagina. The plug, composed of coagulated secretions from male accessory sex glands, resembles a waxy grain of rice. The presence of a plug indicates that coitus and ejaculation have occurred. When a plug is discovered, that female is placed in a cage marked with the date of breeding and is tentatively considered pregnant. The day the plug is observed is considered Day 1 of gestation, unless otherwise noted.

What to Expect for Timed Pregnant Orders

How long is gestation in the rat and mouse? Counting the day the plug is detected as Day 1, parturition (birth) occurs most often on Day 20 in mice and Day 22 in rats. However, variability is normal and will occur between individuals, as well as between species and strains. As a result, not all rats and mice will deliver on the same day.

For untimed pregnant orders, a variation of 2-3 days gestation can be expected. The actual gestation and/or exact day of littering cannot be guaranteed, nor can the number of pups born in a litter.

What day does implantation occur? Implantation occurs about Day 4-5 in mice and Day 5-6 in rats. We recommend that whenever possible, customers avoid orders which would ship animals during the time of implantation.

In both mice and rats, a phenomenon called embryonic diapause is possible. In this condition, the female halts the development of the embryos as hatched blastocysts. They can be held in the uterus for up to 13 days. Although this phenomenon is normally associated with physiological stress of lactation, it is possible that failure or delay of implantation may occur under other stressful conditions. Please see the table below to understand the guarantees that apply for timemated rats and mice.

Are all "plugged" animals pregnant? Not all females in which a plug is observed will become pregnant. However, for timed pregnant rat orders of 13 days or greater in gestation, the stage at which fetuses can be palpated, all rats received by customers should be pregnant.

For orders of less than 13 days in gestation, fetuses are not palpated, and the percentage of pregnant ones will vary with the strain and possibly the stage of gestation at shipping as well. For outbred rats, 90% or more "plugged" females are usually pregnant. In general, a higher percentage of outbred mice or rats will be pregnant than inbred mice or rats. For inbred mice, prior to visual abdominal distention, the percentage of pregnancy is often less than 50%, possibly lower for some strains.

Pregnant Animal Policy

Charles River only uses the most up-to-date techniques and technology in producing pregnant animals for your order. Rats can be safely and accurately palpated after 13 days of gestation. Prior to that, determination of pregnancy (in both rats and mice) is made by the observation of a vaginal plug.

More than 99% of our timed pregnant orders are filled successfully. We strive to continuously improve our ability to prevent all problems that may arise, especially those associated with the more difficult orders, such as inbred mice in early stages of gestation.

How to Report Problems with Charles River Laboratories Pregnant Orders

Charles River stands behind all its orders. Should you experience a problem with your timed pregnant animal order, contact Client Relations at 1-800-522-7287. In order for our specialists to help serve you better, please have the following information ready:

- The actual number of animals received (including any extras)
- · How many were pregnant?
- If the animals were not pregnant, were implantation or resorption sites observed?
- If the animals were pregnant, but the stage of gestation appeared to be incorrect, what day did it appear to be?
- · How was this determined?

This information, coupled with the specifics on your order we'll have on file, will help resolve your problem in a quick, satisfactory manner.

Pregnant Animal Guarantee Policy*

Stock or Strain	Timed Pregnant Up to 12 days gestation	Timed Pregnant 13 days gestation and over	Untimed Pregnant 13-17 days gestation only
Outbred Rats	Plug Guarantee Only	100%	95%
Inbred Rats	Plug Guarantee Only	75%	75%
Outbred Mice	Plug Guarantee Only	100%	100%
Inbred Mice	Plug Guarantee Only	75%	75%

^{*} Weekend timed pregnant orders may incur surcharges. Please call Client Relations at 1-800-522-7287 for details.

