

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.

Most Charles River colonies that provide timed pregnant rats screen females with an Impedance Meter Device (IMD) to determine their stage of estrus prior to using them for breeding. The IMD has a probe that is inserted into the vagina to measure electrical impedance. Due to changes in the composition of the vaginal mucus, rats in proestrus will have higher impedance values than rats in other stages of the estrous cycle. Based upon these measurements, only the female rats determined to be at the appropriate point in the estrous cycle are selected for breeding. The IMD does not affect the probability that an animal will actually become pregnant.

In timed pregnant orders of rats in which the females are shipped at 13 days or more of gestation, pregnancy is confirmed by abdominal palpation. If the female breeder is less than 13 days of gestation the young fetuses are too small to be reliably palpated and might even be damaged by this procedure. Mice are not palpated, but rather the breeder female is observed for abdominal distension to indicate pregnancy. This is usually visible by Day 13 in CD-1[®] and CF-1[®] outbred mice, and by Day 14 in inbred mice and CFW[®] outbred mice. Although less stressful to the fetus than palpation, observation of abdominal distension is not as accurate in verifying pregnancy.

What to Expect for Timed Pregnant Orders

• Are all “plugged” animals pregnant?

No. 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. Nonetheless, when available a few “extras” may be included to assure that the order will

be successfully filled. For orders of less than 13 days in gestation, fetuses are not palpated, and the percent pregnant will vary with strain and possibly the stage of gestation at shipping as well. For CD[®] 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 percent pregnant is often less than 50%, and may even be lower for some strains. In order to compensate for the lower conception rate in inbred mice, “extras” are shipped with the order when available.

• What day does implantation occur?

Implantation occurs about Day 4-5 in the mouse and Day 5-6 in the rat. We recommend that whenever possible, customers avoid orders which would ship animals during the time of implantation.

How Charles River Laboratories Provides Untimed Pregnant Orders

Untimed pregnant rats and mice are also available. Untimed pregnant females are less expensive than timed pregnant females and are available on short notice. These animals are not time mated, nor is the impedance meter used. Untimed pregnant rats are available after 13 days of gestation. However, a 2-3 day range of gestation is mandatory for these 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 various stocks and strains. As a result of this variation, not all rats and mice will deliver on the same day.



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Pregnant Animal Policy

Charles River uses only the most up-to-date techniques and technology in producing pregnant animals for your order, including the use of an impedance meter for determining the stage of estrus cycle in rats. 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. Plug date is considered to be Day 1 of gestation. Charles River cannot guarantee the number of pups per litter.

More than 99% of our timed pregnant orders are filled successfully. We take great pride in our success rate, but overall averages are no consolation to an individual investigator who has experienced a problem with a particular order. Therefore, we are continuously improving 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 of its orders. Should you experience a problem with your timed pregnant animal order, contact Customer Service at 1-800-522-7287. In order for our Customer Service specialists to help serve you better, please be prepared with the following information:

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, together with the information we will already have on file concerning the specifics of your order, will help us to resolve the problem quickly and to your complete satisfaction.

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
All Outbred Rats	90%	100%	100%
Inbred Rats	Plug Guarantee Only	75%	75%
CD-1, CF-1, CFW Mice	75%	100%	100%
Inbred & Specialty Mice	Plug Guarantee Only	75%	75%

Please be aware that weekend timed pregnant orders can incur surcharges.

Please call Customer Service at 1-800-522-7287 for details.