

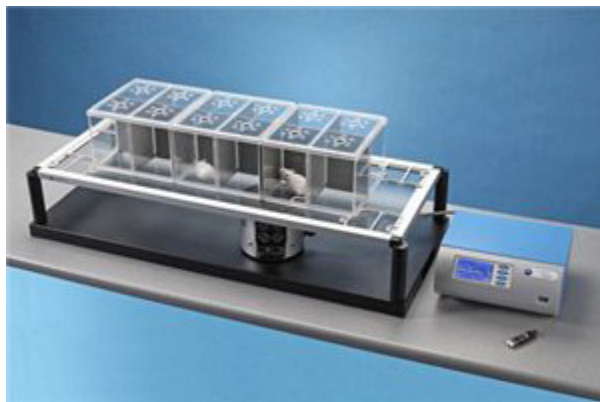
**Hargreaves Test**  
**IACUC Standard Procedure**  
**Effective Date: June 2024**

**Description of Procedure:**

The Hargreaves test is designed to assess thermal pain sensation in rodents. A thermal stimulus, generated by a focused light source, is applied indirectly to the plantar surface of the hind paw of an animal. The response to this stimulus consists of withdrawal of the stimulated paw.

**Procedure Steps:**

1. An animal is placed on a transparent glass surface and a clear container is positioned over the animal with the open end of the container in contact with the glass surface. Movement is limited but the animal is not restrained.
2. After the acclimation period, the infrared (IR) source placed under the glass floor is positioned by the operator directly beneath the hind paw. A trial is started by activating the IR source.
3. When the animal feels pain and withdraws its paw, the IR source switches off and the reaction time counter stops. The withdrawal latency to the nearest 0.1s is automatically determined and recorded.
4. If there is no response up to by 35 seconds, the test is terminated. Temperatures range from 45°C to 52°C.



(Photo credit: Linton Instruments)

**Adverse effects:**

Pain, tissue injury

**References:**

Cheah M, Fawcett JW, Andrews MR. Assessment of Thermal Pain Sensation in Rats and Mice Using the Hargreaves Test. *Bio Protoc.* 2017 Aug 20;7(16):e2506. doi: 10.21769/BioProtoc.2506. PMID: 28920069; PMCID: PMC5600253.