**Preferred site:**
Animal’s lower left abdominal quadrant.

**Needle size:**
21 gauge or smaller (usually use 25 gauge for luciferin injection)

**Volume:**
100ul of luciferin (15mg/ml stock) per 10 grams of mouse body weight.
Note: 1ml i.p. injection of a nonirritating solution is well tolerated.

**Position of animal:**
Manually restrained, dorsal recumbence (abdomen side up), with cranial (head) end of animal pointed down. Gently shake the mouse 2-3 times to encourage the intestinal content downward and to create an empty cavity in the lower abdominal quadrant. Prep the site with 70% ethanol.

**Injection:**
Needle should be bevel-side up and slightly angled 15 - 20 degrees when entering the abdominal cavity. Penetrate just through abdominal wall (about 4-5mm). Aspiration should be attempted (pull back slightly on the needle) to ensure that an abdominal viscus (hollow organ such as the bladder or colon) has not been penetrated. If material is aspirated, the syringe should be removed and disposed. Never inject GI tract contents or urine into the peritoneal cavity, as a bacterial or chemical peritonitis will likely result. The tip of the needle should just penetrate the abdominal wall of the animal’s left lower abdominal quadrant. Slowly inject the luciferin into the intraperitoneal cavity, remove needle and discard safely.

**Tips:**
To ensure accuracy of depth and to inhibit deeper penetration, a piece of catheter tubing can be threaded over the needle leaving the appropriate amount of the needle exposed to assure 4-5mm injection depth.

Half of the volume of your D-luciferin substrate can be injected on one side of the animal and the other half of the volume on the opposite side. However, if proper placement of the needle occurs, this is not necessary.