In this section of the protocol you will provide details about all the procedures that will be performed on the animals you are using. You have two options for adding procedures, either Pre-filled Procedures or Custom Procedure. We also describe how to Clone a Custom Procedure and provide General Notes about all Procedures in this Guide. For some types of Custom Procedures, there may be ACUC-approved procedure descriptions available on the web site, https://www.olac.berkeley.edu/pi-resources/pre-approved-procedures.

1) **Pre-filled Procedure** are auto-populated with ACUC-approved procedure descriptions and procedural steps. There are several types of Pre-filled Procedures, listed below.

<table>
<thead>
<tr>
<th>Rodent Breeding Procedures</th>
<th>Identification under Anesthesia</th>
<th>Identification of Anesthetized Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent Blood &amp; Tissue Collection Procedures</td>
<td>Blood Collection under Anesthesia</td>
<td>Blood Collection in Anesthetized Mice</td>
</tr>
<tr>
<td>Rodent Compound Administration Procedures</td>
<td>Compound Administration under Anesthesia</td>
<td>Compound Administration in Anesthetized Mice (ID, RO, HGD)</td>
</tr>
<tr>
<td>Rodent Compound Administration Procedures</td>
<td>Compound Administration under Anesthesia</td>
<td>Compound Administration in Anesthetized Rats (ID, IM, &amp; IV)</td>
</tr>
<tr>
<td>Rodent Compound Administration Procedures</td>
<td>Compound Administration without Anesthesia</td>
<td>Compound Administration in Conscious Mice (PO, SC, IP, IM, IV)</td>
</tr>
<tr>
<td>Rodent Disease &amp; Immunology Models</td>
<td>Immunization</td>
<td>Immunizations for Antibody Production in Mice</td>
</tr>
<tr>
<td>Rodent Disease &amp; Immunology Models</td>
<td>Hematopoietic Chimerism</td>
<td>Production of Hematopoietic Chimeric Mice</td>
</tr>
<tr>
<td>Rodent Disease &amp; Immunology Models</td>
<td>Skin Grafts</td>
<td>Performing Skin Grafts in Mice</td>
</tr>
<tr>
<td>Rodent Disease &amp; Immunology Models</td>
<td>Tumor Cell Injections</td>
<td>Tumor Cell Injection in Conscious Mice (SC, IP, IV)</td>
</tr>
<tr>
<td>Rodent Disease &amp; Immunology Models</td>
<td>Tumor Cell Injections</td>
<td>Tumor Cell Injection in Conscious Rats (SC, IP, IV)</td>
</tr>
<tr>
<td>Rodent Disease &amp; Immunology Models</td>
<td>Tumor Cell Injections</td>
<td>Tumor Cell Injection in Anesthetized Mice (SC, IP, IV, RO)</td>
</tr>
</tbody>
</table>
Tumor Cell Injections | Tumor Cell Injection in Anesthetized Rats (SC, IP, IV)
---|---
Rodent Surgical Procedures | Stereotaxic compound administration | Stereotaxic Compound Administration in Mice
| Stereotaxic compound administration | Stereotaxic Compound Administration in Rats
Rodent Diagnostic Procedures | Imaging | Imaging Mice (IVIS, Ultrasound, MRI, EchoMRI, & Fluorescence)
| Imaging | Imaging Rats (IVIS, Ultrasound, MRI, & Fluorescence)

a. To add a **Pre-filled Procedure** to your protocol, go to the **Protocol Information**, **Procedures** section of the protocol, select the **Pre-filled** radial button and click **Add**.

b. Using the drop-down menu, select the **Procedure Category**, referring to the **table above** for guidance:
c. Using the drop-down menu, select the **Procedure Type**, referring to the table above for guidance:

![Procedure Type Selection](image)

**Procedure Type Options:**
- Rodent Blood and Tissue Collection Procedures
- Rodent Breeding Procedures
- Rodent Compound Administration Procedures
- Rodent Dissection
- Rodent Surgery
- Other

_d. Using the drop-down menu, select the **Pre-filled Procedure Title**:_

![Pre-filled Procedure Title](image)

**Pre-filled Procedure Titles:**
- Rodent Blood and Tissue Collection Procedures
- Rodent Breeding Procedures
- Rodent Compound Administration Procedures
- Rodent Dissection
- Rodent Surgery
- Other
e. The **Species** and **Pain/Distress Category** will auto-populate based on the procedure:
f. You can edit or change the **Pain/Distress Category** for the procedure, but the **Species** is fixed, depending on the **Procedure Type** you selected.

g. Complete all the other information on the **Procedure Details**, including the maximum number of animals to be used in this procedure for a THREE-YEAR period, use location and, for procedures involving category D or E pain and distress levels, check “Yes”, a veterinarian was consulted (a veterinarian will review the protocol).

h. Click **Save**. The **Procedure Details tabs** will appear and will need to be completed.

i. At this time, you can edit the **Pre-filled Procedure Title**. You should do this if you intend to add more than one of the same Pre-filled Procedure Titles.

i. **Carefully read all the instructions** under **Procedure Description**. You can edit this information if needed, but keep in mind that existing content has been approved by the ACUC. All changes made will be tracked for reviewers.
j. Under **Procedural Steps**, select the box for those steps or methods you will **NOT** be using and click **Delete**. There can be several methods described, so carefully read all the content.

To edit the **Step Description**, click on the link to the **Step Name**. All changes made are tracked for reviewers.

k. To edit the **Step Description**, click on the link to the **Step Name**. **Save** your changes. Keep in mind that the existing content has been approved by the ACUC. Any changes you make will be tracked for reviewers. All changes you make the **Procedure Steps** will be visible once you **Save the Procedure**.
1. Scruff mouse by grasping loose skin over the shoulders between thumb and index finger of non-dominant hand. **You can edit the procedural step description, and these changes will be tracked for reviewers.**

2. Puncture facial vein, located slightly behind the mandible, but in front of the ear canal near the bald spot or “dimple”, in a swift, lancing motion with a 4.0-5.5mm lancet or tip of a 19-25g needle; blood will flow immediately if in the correct location.

3. Collect sample into a pipette via capillary action or allow blood to drip into a microcentrifuge or blood collection tube.

4. Apply pressure with a gauze pad until bleeding stops.

**Potential Adverse Events:** Depth of the puncture must be controlled or excessive bleeding, entry into the ear canal, entry into the oral cavity, hematoma formation, trauma to the underlying muscles or infection can occur.

**Note:** Hemostasis may take longer than other methods of blood collection.

---

I. Review the auto-populated content in the remaining questions on the **Procedure Description** tab, and be sure to answer the question, **“How does this procedure fit into or address your overall research goals?”**

- Answer the question, **“How does this procedure fit into or address your overall research goals?”**
- Review all the auto-populated content in the remaining questions, and edit as needed. All changes made will be tracked for reviewers.
m. To save the procedure and keep it **open** for editing, click the blue **Save Procedure** button within the **Procedure Description**. To save the procedure and **close** the procedure, click the **Save** link in the grey border.

n. **Review all the other procedure details tabs.** There will be auto-populated content in other tabs, depending on the procedure type.
<table>
<thead>
<tr>
<th>Procedure Description</th>
<th>Anesthetic Regimen</th>
<th>Other Agents Utilized</th>
</tr>
</thead>
</table>

Procedure Description

Please make sure that your purpose for performing this procedure is fully described below in the section "How does this procedure fit into or address your overall research goals?"
o. If you are using Anesthesia there are several **Anesthetic Agent** options auto-populated. Delete any you will not be using by **clicking on the box** next to the agent and clicking the **Delete** link.

- **Add** all personnel who will be performing anesthesia for this procedure.

Carefully review **Parameters** that will be monitored. Make edits if needed.

Carefully read **Anesthetic Recordkeeping Methods**.

- **Add** **Paralytic Agents** or Other **Pre-medications** if used.

Carefully read **Anesthetic Doses**. Edit, Delete or Add as needed.

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### Anesthetic Regimen

For guidance, please refer to the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

#### Parameters that will be monitored during anesthesia (check all that apply):

- [ ] Respiratory Rate
- [ ] Heart Rate
- [ ] Body Temperature
- [ ] Blood Pressure
- [ ] Corneal/Palpebral Reflex
- [ ] Pedal Reflex
- [ ] Capillary Refill
- [ ] PO2
- [ ] ETCO2
- [ ] Other (Describe):
  - Skin color or mucous membrane color

#### Describe Anesthetic Recordkeeping Methods.

<table>
<thead>
<tr>
<th>Agent Name</th>
<th>Dosage (in mg/kg if possible) and Route</th>
<th>Add</th>
<th>Delete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>Induce 3-4%, Maintain 1-2% Inhilation (IN)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine hydrochloride</td>
<td>80-100 mg/kg Intrasminal (IP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylocaine</td>
<td>5-10 mg/kg Intrapertoneal (IP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>1-2 drops to effect Inhilation (IN)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For guidance, please refer to the ACUC Guidelines for the Use of Neuromuscular Blocking Drugs in Anesthetized Animals.

<table>
<thead>
<tr>
<th>Paralytic Agents</th>
<th>Add</th>
<th>Delete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldenuron</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For guidance, please refer to the ACUC Peri-procedure Care Tab.

<table>
<thead>
<tr>
<th>Other premedications not already listed above</th>
<th>Add</th>
<th>Delete</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
p. If you will be administering any material or agent to the animals as a part of this procedure (other than Anesthetic or Analgesics already described), open and complete the Other Agents Utilized tab.

q. Once you save and close a pre-filled procedure, it will appear on the Procedures list page, and will be identified as a pre-filled procedure:

r. **PLEASE NOTE:** The Clone function is not available for Pre-filled Procedures, only for Custom Procedures.
2) **Custom Procedure** do not include any auto-populated content. There are several types of custom procedures that you can add, and selecting the correct type of procedure is important since this dictates the questions the Investigator must respond to when describing the procedure. For some types of Custom Procedures, there may be ACUC-approved procedure descriptions available on the web site, [http://www.olac.berkeley.edu/pi-resources/pre-approved-procedures](http://www.olac.berkeley.edu/pi-resources/pre-approved-procedures). The **Custom Procedure Types** are listed below:

- Animal Identification
- Antibody Production
- Behavior Study Assay
- Blood Collection
- Breeding & Genotyping
- Compound/Drug Administration
- Experimental Neoplasia/Tumor Passage
- Field Studies
- Imaging
- Infectious Disease Study
- Irradiation/Reconstitution
- Neurobiological Recording
- Nutritional Study
- Surgery at Vendor
- Surgical Procedure
- Treadmill/Locomotion
- Other (describe)

a. To add a custom procedure to your protocol, go to the **Protocol Information, Procedures** section of the protocol, select the **Custom** radial button and click on the **Add** link.

b. You can also select an existing custom procedure and **Clone** it. Cloning is helpful if you are doing the same procedure on multiple species.

i. **PLEASE NOTE:** The Clone function is not available for Pre-filled Procedures, only for Custom Procedures.

ii. To clone an existing custom procedure, **select the box** next to the procedure you wish to clone and click the **Clone** link (see screen shot below).

iii. If you **Clone a procedure**, you will need to give the new procedure a **unique Procedure Title**.
c. After you click Add or Clone, a **Procedure Details** section will appear:

To Clone an existing Custom Procedure, select it, then click Clone.

After you click Add or Clone, a Procedure Details section will open.
3) Select the **Procedure Type** from the drop-down menu, or select **Other** and describe.

4) Complete the **Procedure Title**, distinguishing each procedure. For example, if you will be doing several types of Surgical Procedures, use the title to distinguish one surgical procedure from another. This is helpful to both reviewers and researchers, so they can easily drill-down to the details of the different procedures in the protocol.

5) Select the species from the drop-down menu, which includes all the species you added previously in the **Species** section of the protocol.

6) The **Pain and Distress Category** automatically selects a default setting based on the Procedure Type selected. You can change the default setting, however, the official Pain and Distress Category will be determined by the ACUC during the review process.

d. The **Pain and Distress Category** is required for regulatory reporting purposes and uses a scale developed by the United States Department of Agriculture (USDA), the agency that administers the Animal Welfare Act Regulations.

i. **Category B**: Animals that will be bred or purchased for breeding, but not used for experiments. This includes:
   - breeders
   - young that cannot be used because of improper genotype or gender
   - any other animals that will not have any research procedures performed on them or participate in research studies
   Note: If tail snips are necessary for genotyping, this category is **not** appropriate.

ii. **Category C**: Animals that will undergo procedures that involve no or only very brief pain or distress, with no need for or use of pain relieving drugs. Examples include:
   - observational studies
   - most intravenous and parenteral injections of non-irritating agents
   - most blood collections from peripheral vessels
• collection of cells and/or tissues from animals after euthanasia has been performed

iii. **Category D**: Animals that will undergo procedures involving potential pain or distress *that is relieved* by appropriate anesthetics, sedatives, or analgesics. Examples include:
  • major and minor surgery performed under anesthesia (survival or non-survival)
  • tissue or organ collections prior to euthanasia
  • painful procedures performed under anesthesia (such as retro-orbital blood collection in rodents)
  • prolonged restraint accompanied by tranquilizers or sedatives
  • experiments involving infectious or other hazardous materials in animals that have provisions for immediate euthanasia if they become sick to effectively prevent pain and/or suffering.
  
  Note: If an endpoint is used that involves significant pain or distress, consideration should be given to putting animals into Category E.

iv. **Category E**: Animals that will undergo procedures in which pain or stress is NOT relieved with the use of anesthetics, analgesics, tranquilizers, or by euthanasia. Examples include:
  • studies in which animals are allowed to die without intervention (e.g., LD50, mortality as an end-point)
  • studies that allow endpoints that are painful or stressful
  • addictive drug withdrawals without treatment
  • pain research or noxious stimulation

7) Complete the **maximum number of animals** to be used in this procedure for a **three-year period**.

8) If you have added a Category D or E procedure, you will be asked to certify that you have **consulted with one of the OLAC veterinarians**. Click “Yes” in response to this question. *(It is recommended to consult with veterinarians on procedures prior to submission, however, veterinarians review all protocols submitted to the ACUC).*

9) Complete the **Procedure Site Information**, including the building and room number.

e. This information is used to determine if the space is approved by the ACUC for the procedure listed and to assist the ACUC in fulfilling its regulatory obligation to inspect all areas where animals are used.

   **NOTE:** If you change the location where you perform a procedure, you must **amend your protocol** so the Procedure Site information is up-to-date.

f. If you are performing the same procedure in multiple locations, you will need to add a separate procedure for each location. You can create one procedure and **Clone** it to change the procedure site.

g. If you are conducting field studies, select **Use Location**: “Field Study”, **Building**: “Other”, and type “NA” into the Building and Room fields. You will provide more detail about where your field studies are conducted later in the procedure description.
10) When you have completed all the required **Procedure Details** information, click the **Save** button.

11) Depending on the **Procedure Type** selected, additional questions will appear in blue tab format under the **Procedure Details**.

h. Complete all required and applicable questions on each tab for each procedure. All questions marked with a red asterisks (*) must be completed before moving on.

i. Click on the **Next** button under the Procedure Detail tabs to save your work and move to the next tab.

j. If a question is not applicable, please type “Not Applicable” or “NA” rather than leaving it blank.

12) Repeat steps 2-11 to add every procedure to your protocol. eProtocol will build a table in the Procedure Section of the Protocol that lists all of your procedures.

k. To edit a procedure, click on the **Procedure Type** link.

l. To delete or clone a procedure, select the box next to the procedure and click **Delete** or **Clone**.
3. **General Notes about all procedures:**

   c. **NAVIGATION TIPS:** At this stage of the protocol, there are multiple navigation tools for saving and proceeding.

   i. The **Next** and **Save** links **below** the blue Procedure Details Tabs save each tab for **that procedure**.

   ii. The **Save** link **next to** the words, “**Procedure Details,**” will save **and close** that procedure.

   iii. The **Next** and **Previous** links at the top right-hand side of the page will save and move you to the next section of the protocol (e.g., Funding Sources or Certifications).

   iv. The **Save** and **Close** links at the top right-hand side of the page will save and close the protocol itself.

![Navigation Tips Diagram]
d. If you add more than one procedure, you must complete the **Procedure Relationships** section. This section describes your studies (which consist of multiple procedures), the sequence and timing of the procedures, and **how many and which procedures a single animal will undergo**. See the [eProtocol Quick Guide on Procedure Relationships](#).
e. If you add a potentially painful or distressful procedure (see Pain and Distress Category, above), you must complete the Alternatives Search section, describing in detail how you performed a literature search for alternatives to the procedure. See the eProtocol Quick Guide on Alternatives Search.

f. If you are performing the same procedure on different species, you will need to add a separate procedure for each species.