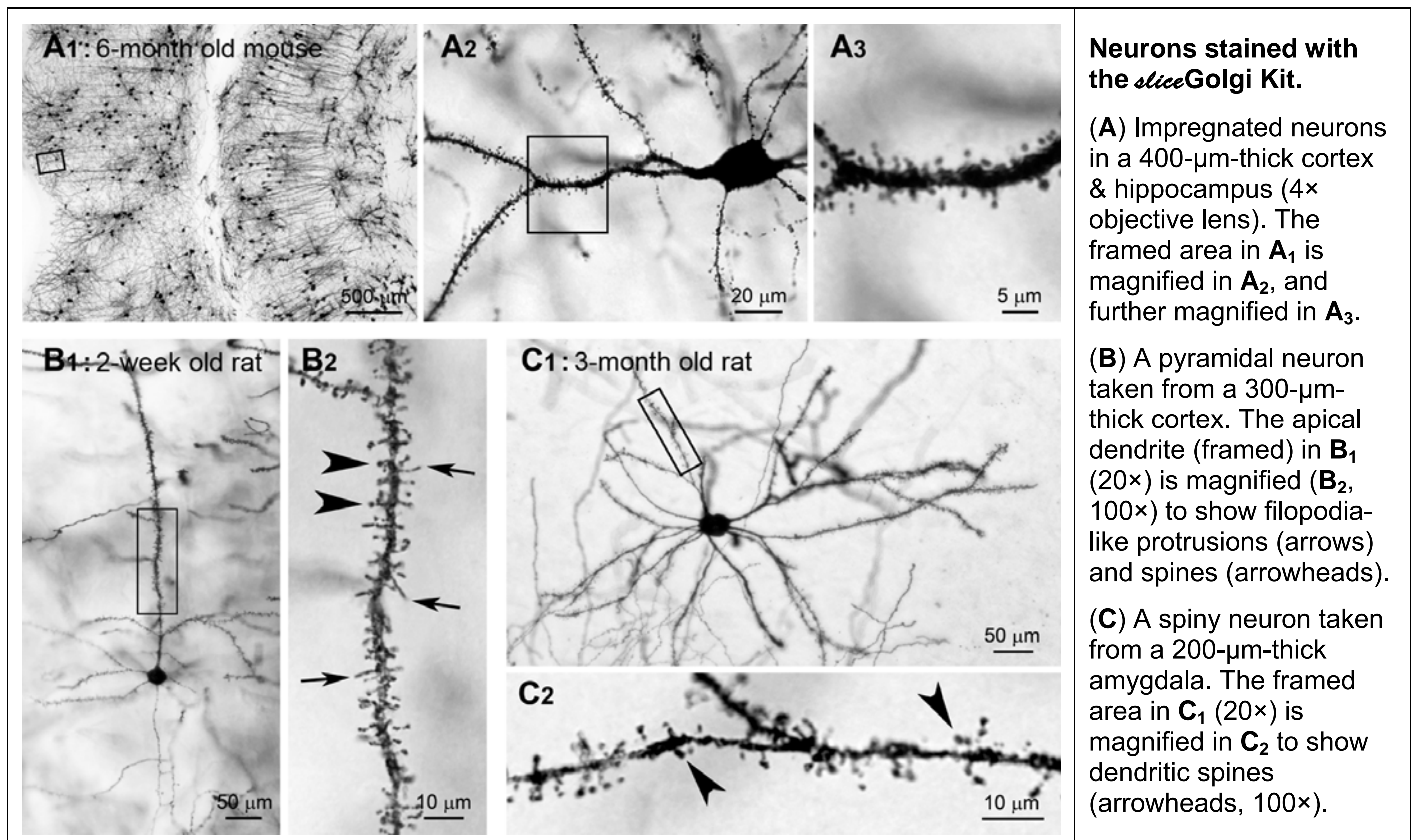
PERFORM GOLGI-STAINING ON SLICES/SECTIONS (LABORATORY USE ONLY)

The *slice*Golgi Kit is designed for running Golgi-staining on **slices/sections** (see references). The impregnation and staining of neurons can be performed on **free-floating sections** (50~400 μm thickness). The kit has been extensively tested across many types of brain sections from both rats and mice including (1) freshly harvested sections, *e.g.*, acute slices quickly prepared using tissue chopper; (2) organotypic slice cultures, *e.g.*, hippocampal slice cultures and cortical slice cultures; (3) artificial cerebrospinal fluid (ACSF)-infused brain slices that have completed electro-physiological recording and/or drug administration; and (4) sections derived from brains fixed with Bioenno Fixative (*Catalog Number: 003780*) via either perfusion or immersion. The resulting outcome includes both reliable and high quality staining of dendritic branches and spines of neurons. The kit can be stored in a dark area at room temperature (15-25ºC) for up to 12 months. The *slice*Golgi Kit may be applied to retina, spinal cord, and cultured cells to stain the neuronal processes. The kit does not work for frozen tissues.



Neurons stained with the *slice*Golgi Kit.

(**A**) Impregnated neurons in a 400-μm-thick cortex & hippocampus (4× objective lens). The framed area in $\mathbf{A_1}$ is magnified in $\mathbf{A_2}$, and further magnified in $\mathbf{A_3}$.

(**B**) A pyramidal neuron taken from a 300-μm-thick cortex. The apical dendrite (framed) in $\mathbf{B_1}$ (20×) is magnified ($\mathbf{B_2}$, 100×) to show filopodia-like protrusions (arrows) and spines (arrowheads).

(**C**) A spiny neuron taken from a 200-μm-thick amygdala. The framed area in $\mathbf{C_1}$ (20×) is magnified in $\mathbf{C_2}$ to show dendritic spines (arrowheads, 100×).

References:

- Harris KM, Cruce WL, Greenough WT, Teyler TJ. A Golgi impregnation technique for thin brain slices maintained *in vitro*. J Neurosci Methods 1980; 2:363–371.
- Gabbott PL, Somogyi J. The 'single' section Golgi-impregnation procedure: methodological description. J Neurosci Methods 1984; 11:221–230.
- Frotscher M. Application of the Golgi/electron microscopy technique for cell identification in immunocytochemical, retrograde labeling, and developmental studies of hippocampal neurons. Microsc Res Tech 1992; 23:306–323.
- Angulo A, Fernández E, Merchán JA, Molina M. A reliable method for Golgi staining of retina and brain slices. J Neurosci Methods 1996; 66:55–59.
- Levine ND, Rademacher DJ, Collier TJ, O'Malley JA, Kells AP, San Sebastian W, Bankiewicz KS, Steece-Collier K. Advances in thin tissue Golgi-Cox impregnation: fast, reliable methods for multi-assay analyses in rodent and non-human primate brain. J Neurosci Methods 2013; 213:214–227.

Warranty: Warranty on the items in the kit is 12 months from the date of purchase.
Return Policy: Bioenno Tech's return policy for this product is 90 days from the date of purchase.
Free Technical Support: Email your questions to contact@bioenno.com

MATERIALS PROVIDED WITH THE KIT:

- **Stock Solutions A1, A2, and A3** are designed for the preparation of 500 ml **Fixative**.

  A1 (125 ml × 1 QTY): Buffer Stock Solution containing Na^+, K^+, and Ca^{++}. pH 7.4 ± 0.1.
  A2 (125 ml × 1 QTY): Formaldehyde Stock Solution.
  A3 (25 ml × 1 QTY): Glutaraldehyde Stock Solution.

  Prepare the aldehyde **Fixative** immediately before use (20 ml as an example):
  1) To 9.0 ml of distilled or deionized water (dH_2O), add 5.0 ml of **A1 Buffer** Stock Solution and mix well;
  2) Add 5.0 ml of **A2 Formaldehyde** Stock Solution and mix well;
  3) Add 1.0 ml of **A3 Glutaraldehyde** Stock Solution and mix well, to obtain 20 ml of fixative.

- **Stock Solution B** (250 ml × 1 QTY): The 5× stock solution is intended for the preparation of the **Impregnation Solution**. It is normal to see precipitate on the bottom, simply use the supernate.

  The 5× stock solution must be diluted with dH_2O (1:4 by volume) before use (*e.g.*, to 5.0 ml of **Stock Solution B**, add 20 ml of dH_2O, and mix well to generate 25 ml of **Impregnation Solution**).

- **Solution C** (250 ml × 1 QTY): **Staining Solution** (working solution, use it directly).

- **Solution D** (250 ml × 1 QTY): **Post-staining Solution** (working solution, use it directly).

- Also included: Shader Paintbrush and Round Paintbrush (1 QTY each).

Materials necessary but NOT included:

Distilled water and/or deionized water (dH_2O);
0.1 M PB and 0.01 M PBS-T (see the following **Table**);
Plastic/glass tubes, culture plate wells, and bottles;
Adhesive microscope slides, coverslips, ethanol, xylene, and Permount® mounting medium.

Table: To prepare 0.01 M PBS-T, first prepare 0.1 M PB (Left), and then PBS containing 0.3% Triton X-100 (Right).

0.1 M PB, pH 7.4	1,000 ml
$NaH_2PO_4 \cdot H_2O$	2.62 g
$Na_2HPO_4 \cdot 7H_2O$	21.73 g
add dH_2O to	1,000 ml
(stir to enhance dissolution)	

0.01 M PBS-T, pH 7.4	1,000 ml
NaCl	8.5 g
0.1 M PB (pH 7.4)	100 ml
Triton X-100	3 ml
add dH_2O to	1,000 ml
(stir and heat to 50-55ºC to enhance the dissolution of Triton X-100)	

STORAGE, SAFETY, AND HANDLING PRECAUTIONS:

- Store the kit at room temperature (15-25ºC) or at 4°C, but not in a freezer.
- Solutions **A**, **B**, and **C** in the kit contain toxic reagents. Prepare and use them in a fume hood. Collect any waste from solution **A**, **B**, and **C** in a bottle for hazardous waste disposal.
- Wear gloves, appropriate eye and face protection, and suitable protective clothing while handling kit reagents. Wash hands thoroughly after handling.
- Avoid inhalation and contact with skin and eyes while handling. In case of contact, wash immediately and thoroughly with water and seek medical advice if necessary.

PROTOCOL:

- Use glass or plastic container with cover. Do not use metal tools to attach **Impregnation** and **Staining Solutions**.
- Protect the slices/sections from light while treating them with **Impregnation Solutions** and **Staining Solution**.
- Perform the experiments at room temperature (15-25ºC, RT) for the best results, and do not re-use the diluted/working solution.

1. **Fix slices/sections:**

   a. Prepare aldehyde **fixative** immediately before use, as follows (20 ml as an example):

   (1) To 9.0 ml of dH_2O, add 5.0 ml of **A1 Buffer** Stock Solution and mix well;
   (2) Add 5.0 ml of **A2 Formaldehyde** Stock Solution and mix well;
   (3) Add 1.0 ml of **A3 Glutaraldehyde** Stock Solution and mix well, to obtain 20 ml of Fixative.

   b. Immerse slices/sections into the **fixative** at room temperature (15-25°C) in the dark. The volume of Fixative should be approximately 10 times the volume of the slices/sections.

   c. Renew the **fixative** after 30 min of immersion and continue the fixation for total 3-4 hours, followed by washing slices/sections in 0.1 PB for 1-2 hours (change PB solution 3-4 times during the wash).

   Slices/sections can be stored in the fixative at 4°C for a couple of days. The ideal time for fixation should be obtained by the investigator. Fixed slices/sections can be stored in 0.1 M PB (pH 7.4) at 4°C for up to one week.

   Notes:

   - The above **fixative** can be used to perfuse animals, and the perfused brains can be sectioned at 50-200 µm thickness using a vibratome, followed by **free-floating impregnation**. Briefly, anesthetized animals are first perfused intracardially or via the ascending aorta with 0.9% saline (*i.e.*, 9 g NaCl in 1,000 ml dH_2O) until the visceral blood volume is flushed out, which usually takes 2~5 min, and then perfused with freshly prepared **fixative** for 20~25 min. Brains are dissected from the skull, postfixed in the same fixative for 2~6 hours at RT or overnight at 4ºC, followed by slicing at RT. Sections can be collected and stored in 0.1 M PB (pH 7.4). These fixed sections can directly go to **Step 2**.

   - Additional **Fixative Kit** (2,000 ml of fixative) (Catalog Number: **003780**) allows for perfusion/processing of more samples.

2. **Free-floating impregnation:**

   Immerse slices/sections in **Impregnation Solution** prepared from the **5× Stock Solution** (dilute the Stock Solution with dH_2O, 1:4 by volume). Keep the slices/sections in the Impregnation Solution for 3-7 days at room temperature (15-25°C) and in the dark. Change the solution after 1 day.

   The impregnation is recommended to be performed in 5-15 ml plastic/glass tube or bottle with cover. The volume of impregnation solution should be 10-20 times the volume of the slices/sections.

   Keep the solution and reaction container in the dark, and avoid solution's long-time exposure to the air. The temperature, volume, and pH value of the solution may affect impregnation quality. Variations in type and thickness of sections may affect the time of impregnation and over-impregnation may result in non-specific staining. The optimal time should be determined by the investigator.

3. **Staining and post-staining:**

   a. Once impregnation is complete, briefly rinse the slices/sections in dH_2O, followed by washing in 0.01 M PBS-T for 5 min × 2-3 times.

   b. Transfer the slices/sections to **Solution C** and stain for 2-8 min, followed by brief washing in 0.01 M PBS-T or dH_2O (less than 1 min).

   c. Treat the slices/sections in **Solution D** for 1-4 min, followed by washing in 0.01 M PBS-T for 5 min × 3-4 times.

The staining and post-staining can be performed in 6-well culture plate, and the optimal time for the staining and post-staining should be determined by the investigator.

4. **Mount slices/sections:** Mount sections upon adhesive microscope glass slides (*e.g.,* gelatin-coated slides) in 0.01 M PBS-T. Air-dry sections at room temperature (long-time exposure to dry air may result in fragmented sections). When the sections are dry, go to **Step 5** or store the slides in a dry and closed staining jar (1 day).

   Optional: The mounted slices/sections can be counterstained with cresyl violet, methyl green, or other suitable staining reagent. Wash the mounted sections in PBS-T buffer for 5-10 min, and then perform the counterstaining.

5. **Clean and Cover:** Dehydrate slices/sections in graded ethanols (if the slices/sections are fully air-dried, directly go to 100% ethanol for 5-10 min × 3 times). Clear in xylene or xylene substitute for 5-10 min × 2-3 times. Cover slip using the Permount® mounting medium. Store the sections at room temperature and in a dark area.

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Proven results: The sliceGolgi Kit was used to perform Golgi-staining alone as well as combined Golgi-staining and immunohistochemistry/immunocytochemistry (ICC) on free-floating sections.

A-C: The **sliceGolgi Kit** was used to impregnate and stain neurons in 100 – 200 micron thick, **free-floating sections** of postnatal day 12 (P12) C57BL mouse. (**A**) a stained neuron in the lateral hypothalamic area with numerous filopodia-like protrusions (arrowheads); (**B**) a neuron in the frontal cortex with mature spines (arrows) and filopodia (arrowheads); and (**C**) the main (stem) and oblique dendrites of a pyramidal neuron in the motor area of the frontoparietal cortex.

D-E: The combination of **Golgi-staining** and **ICC** allows simultaneous visualization of dendritic spines and immuno-reactive products. Golgi-staining was first performed on 50 – 100 micron thick, free-floating sections, followed by ICC. The impregnation time was 2 – 4 days at 22 ± 1ºC. Images were taken from the subiculum of a 4-month old C57 mouse (**D**) and from the frontoparietal cortex (somatosensory area) of a 2-month old C57BL mouse (**E**). Boxed areas in **E1** were magnified (63×) (**E2**) to highlight the dendritic spines (arrows) and immuno-labeled neurons (arrowheads).