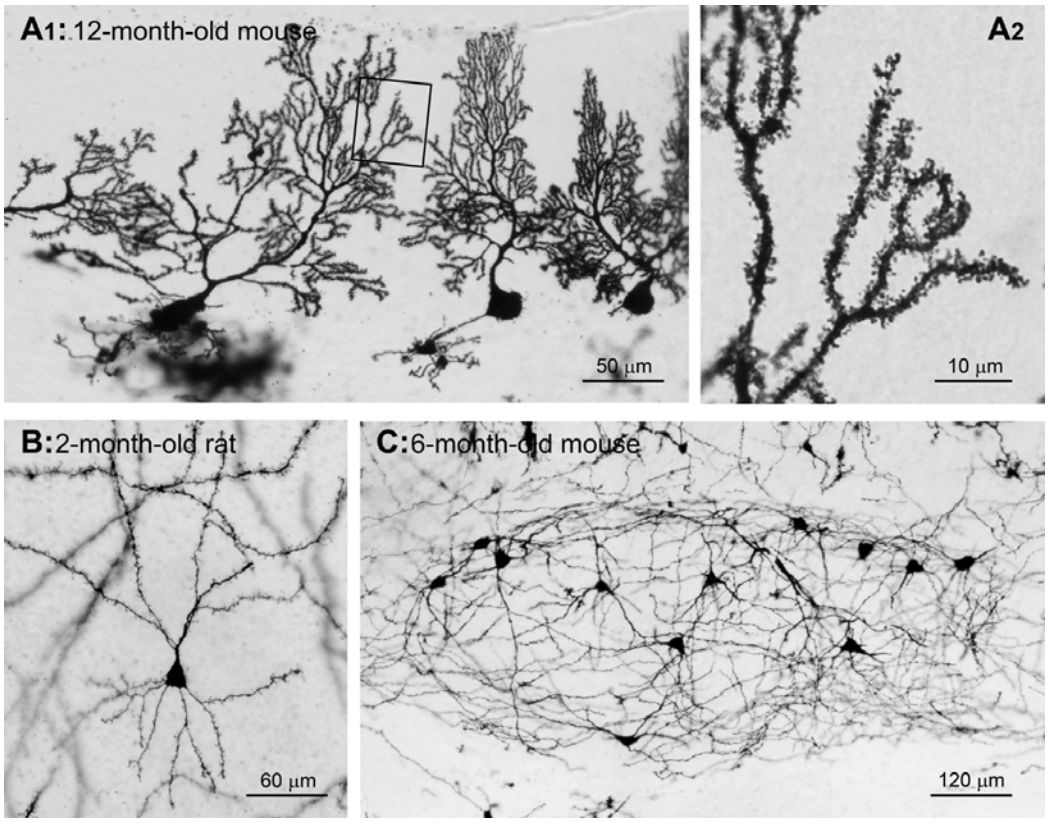
GOLGI-STAINING KIT EXCELLENT FOR FRESH TISSUES (LABORATORY USE ONLY)

Bioenno *elite*Golgi Kit is ideal for staining neurons in the olfactory bulb, cortex, cerebellum, brain stem, and spinal cord (see Figures A-C and visit www.bioenno.com). This kit supplements and extends the capability of our well-received *super*Golgi Kit (Cat #: 003010), and can be used in combination with the *super*Golgi Kit to achieve a more comprehensive analysis of various areas of the brain. The *elite*Golgi Kit has been rigorously tested and validated on brain tissues freshly harvested from rats and mice (It is noted that the kit does not work well on frozen tissues). The impregnation of neurons is rapid and generally takes 3-6 days depending on the age and size of the tissues. The staining of impregnated neurons can be performed on either **free-floating** or **mounted** sections (50~200 µm thickness). The kit can be stored in a dark area at 4-25ºC for up to 12 months.



Neurons stained with Bioenno *elite*Golgi Kit:

(**A**) Impregnated Purkinje cells in the cerebellum (20× objective lens, 200-µm-thick sections). The framed area in $\mathbf{A_1}$ is magnified in $\mathbf{A_2}$.

(**B**) A neuron taken from the primary olfactory cortex (20× objective lens, 200-µm-thick section). Numerous spines on the apical and basal dendritic branches.

(**C**) Impregnated neurons in the hypoglossal nucleus in the medulla. Neurons taken from a 200-µm-thick section at 10× objective lens.

References:

- Ramón-Moliner E: The Golgi-Cox technique. In Nauta WJH and Ebbesson SOE (eds.), Contemporary Methods in Neuroanatomy. pp 32–55, New York: Springer, 1970.
- Glaser ME and Van der Loos H: Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi-Nissl stain. J Neurosci Methods 1981, 4:117–125.
- Kolb B, Ladowski R, Gibb R, Gorny G. Does dendritic growth underlie recovery from neonatal occipital lesions in rats? Behav Brain Res 1996, 77:125–133.
- Gibb R and Kolb B. A method for vibratome sectioning of Golgi-Cox stained whole rat brain. J Neurosci Methods 1998, 79:1–4.
- Orlowski D and Bjarkam CR. Autometallographic enhancement of the Golgi-Cox staining enables high resolution visualization of dendrites and spines. Histochem Cell Biol 2009, 132:369–374.
- Zhang B, Li A, Yang Z, Wu J, Luo Q, Gong H. Modified Golgi-Cox method for micrometer scale sectioning of the whole mouse brain. J Neurosci Methods 2011, 197:1–5.

Free Technical Support: Email your questions to contact@bioenno.com

MATERIALS PROVIDED WITH THE KIT (SAMPLE SIZE):

- Solution **A1** and Reagent **A2** are designed for the preparation of total 60 ml of **Impregnation Solution**.

  A1: **Base Solution**, 30 ml × 1 QTY.

  A2: **Enhancer**, 4.2 g × 1 QTY. Dilute reagent **A2** with distilled or deionized water (dH_2O) before use. Add 28 ml of dH_2O to the bottle and dissolve the reagent to obtain 30 ml of solution **A2**.

  Prepare the **Impregnation Solution** immediately before use (10 ml as an example):

  1) Take out 5 ml of **A1** Base Solution;
  2) Take out 5 ml of **A2** Solution (First dilute the reagent **A2** in dH_2O to obtain 30 ml of solution **A2**).
  3) Mix 5 ml of solution **A1** and 5 ml of solution **A2** (1:1 by volume) to obtain 10 ml of **Impregnation Solution**.

- Solution **B**: **Staining Solution**, 30 ml × 1 QTY. The solution is a working solution, and can be uses directly.
- Solution **C: Clarity Solution**, 30 ml × 1 QTY. The solution is a working solution, and can be used directly.

The stock solutions and reagent are contained in convenient dropper bottles. 1 drop of solution is equal to approximate 40 microliters.

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, bottles, shader paintbrush, and round paintbrush;
Gelatin-coated 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
$NaH_2PO_4 \bullet H_2O$	2.62 g
$Na_2HPO_4 \bullet 7H_2O$	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 in a refrigerator (4°C) or at room temperature (4-25ºC is okay), but not in a freezer.
- Solutions **A1** and **B** in the kit contain toxic reagents. Prepare and use them in a fume hood. Collect any waste from solution **A1** and **B** 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 brain tissues or sections from light while treating them with **Impregnation Solution**.
- Perform the experiments at room temperature (15-25ºC, RT) for the best results, and do not re-use the working solution.

1. Impregnation:

a. Prepare the **Impregnation Solution** immediately before use, as follows (10 ml as an example):

- Take out 5 ml of **A1** Base Solution;
- Take out 5 ml of **A2** Solution (First dilute the reagent **A2** in 28 ml dH_2O to obtain ~30 ml of solution **A2**. *The solution **A2** can be stored at 4ºC for up to 2 weeks.*)
- Mix 5 ml of solution **A1** and 5 ml of solution **A2** (1:1 by volume) to obtain 10 ml of **Impregnation Solution**.

b. Immerse freshly harvested brain tissues into the **Impregnation Solution** at room temperature (15-25ºC) in the dark. The volume of Impregnation Solution should be approximately 5 times the volume of the tissue block. For example, ~5 ml of Impregnation Solution would be the appropriate volume for an adult mouse brain (~1cm × 1cm × 0.5 cm).

c. Renew the solution at the second day, and continue the impregnation for another 2-5 days (total **3-6 days** depending on the age and size of the tissues) at room temperature in the dark.

The ideal time of impregnation should be established by the investigator. If impregnation exceeds 1 week, there could be non-specific staining.

Notes:

For optimal impregnation of neurons, it is recommended to perfuse the animal with saline to flush out blood from the tissue. Anesthetize the animal (such as with sodium pentobarbital, 100 mg/kg intraperitoneally), perfuse 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. The perfusion may take 2~5 min. Dissect the brain and remove from the skull with extreme caution. Block the brain with a sharp blade to approximately 1 cm thickness. Rinse briefly in 0.9% saline, followed by immersing in the Impregnation Solutions.

2. Sectioning:

Sections that are 50-200 µm in thickness can be cut using a vibratome or similar type of microtome. Sections can be collected in 0.1 M PB (pH 7.4, see **Table** on page 2).

A vibratome or micro slicer is recommended for the sectioning.

Trim the impregnated tissue block and glue it to the specimen mount with “super glue”, such as Krazy Glue or Super Glue.

A thickness of 100-200 µm is suggested for analyses of dendritic branches and spines. For thin section cutting (e.g., 50 µm), immerse the razor blade in xylene for 1-2 min to remove any traces of oil.

3. Free-floating Staining and Clarity:

a. Once sectioning is complete, wash sections in 0.01 M PBS-T (see **Table** on page 2) for 5 min × 2-3 times.

b. Transfer the sections to solution **B** (**Staining Solution**) 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 sections in solution **C** (**Clarity Solution**) for 1-4 min, followed by washing in 0.01 M PBS-T for 5 min × 2-3 times.

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

Alternatively, the Staining and Clarity can be performed on mounted sections (may require extra solutions). Briefly, mount sections upon gelatin-coated adhesive slides in 0.01 M PBS-T, and air-dry sections in the dark at room temperature (minutes to hours), followed by Batch or Individual Slide Staining/Clarity.

Batch: Put the Staining or Clarity solution in a staining jar, and add slides and incubate in a dark area at RT for 2-20 minutes depending on the thickness of the sections. After incubation, remove slides and rinse off excess Staining/Clarity solution with 0.01 M PBS-T.

Individual Slide: Place a slide on a level surface and apply the Staining or Clarity solution to sections. Make sure the sections are fully covered with the solution. Incubate the sections in a dark area at RT for 2-20 minutes. After incubation, wash out extra Staining or Clarity solution with 0.01 M PBS-T.

(The optimal time for the staining/clarity should be determined by the investigator.)

*At the end of Clarity, wash the sections/slides in 0.01 M PBS-T for 5 min × 2-3 times, air-dry the sections, and go to **Step 5**.*

4. Mount 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 sections can be counterstained with cresyl violet, methyl green, neutral red, 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 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 and dry area.