

Materials List for:

Measuring Synaptic Vesicle Endocytosis in Cultured Hippocampal Neurons

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URL: <https://www.jove.com/video/55862>

DOI: [doi:10.3791/55862](https://doi.org/10.3791/55862)

Materials

| Name | Company | Catalog Number | Comments |
|--|--------------------|--------------------------------|---|
| Lipofectamine LTX with Plus | Thermo Fisher | 15338-100 | Transfection of plasmid DNA including synaptophysin or VAMP2-pHluorin |
| neurobasal medium | Thermo Fisher | 21103-049 | Growth medium for neuron, Warm up to 37°C before use |
| B27 | Thermo Fisher | 17504-044 | Gradient for neuronal differentiation |
| Glutamax | Thermo Fisher | 35050-061 | Gradient for neuronal culture |
| Poly-D-Lysine coated coverslip | Neuvitro | GG-25-pdl | Substrate for neuronal growth and imaging of pHluorin |
| Trypsin XI from bovine pancrease | Sigma | T1005 | Neuronal culture-digest hippocampal tissues |
| Deoxyribonuclease I from bovine pancreas | Sigma | D5025 | Neuronal culture-inhibits viscous cell suspension |
| pulse stimulator | A-M systems | model 2100 | Apply electrical stimulation |
| Slotted bath with field stimulation | Warner Instruments | RC-21BRFS | Apply electrical stimulation |
| stimulus isolation unit | Warner Instruments | SIU102 | Apply electrical stimulation |
| lubricant | Dow corning | 111 | pHluorin imaging-seal with coverslip and imaging chamber, avoid leak from chamber |
| AP5 | Tocris | 3693 | Gradient for normal saline, selective NMDA receptor antagonist, inhibit postsynaptic activity which have potential for recurrent activity |
| CNQX | Tocris | 190 | Gradient for normal saline, competitive AMPA/kainate receptor antagonist, inhibit postsynaptic activity which have potential for recurrent activity |
| illuminator | Nikon | C-HGFI | Metal halide light source for pHluorin |
| EMCCD camera | Andor | iXon3 | pHluorin imaging, detect pHluorin fluorescence intensity |
| Inverted microscopy | Nikon | Ti-E | Imaging for synaptophysin or VAMP2 pHluorin transfected cells |
| NIS-Elements AR | Nikon | NIS-Elements Advanced Research | Software for imaging acquisition and analysis |
| Igor Pro | WaveMetrics | Igor pro | Software for imaging analysis and data presentation |
| imaging chamber | Warner Instruments | RC21B | pHluorin imaging, apply field stimulation on living cells |

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|-----------------------------|------------------------------|----------|--|
| poly-L-lysine | Sigma | P4832 | Electron microscopy, substrate for neuronal growth, apply on multiwell plate for 1 h at room temperature then wash with sterilized water 3 times |
| Horseradish peroxidase(HRP) | Sigma | P6782 | Electron microscopy, labeling of endocytosed synaptic vesicles by catalyzing DAB in presence hydrogen peroxide, final concentration is 5 mg/mL in normal saline, make fresh before use |
| Na cacodylate | Electron Microscopy Sciences | 12300 | Electron microscopy, buffer for fixatives and washing, final concentration is 0.1 N |
| 3,3'-Diaminobenzidine(DAB) | Sigma | D8001 | Electron microscopy, labeling of endocytosed synaptic vesicles, substrate for HRP, final concentration is 0.5 mg/mL in DDW and filtered, make fresh before use |
| Hydrogen peroxide solution | Sigma | H1009 | Electron microscopy, labeling of endocytosed synaptic vesicles by inducing HRP-DAB reaction, final concentration is 0.3% in DDW, make fresh before use |
| glutaraldehyde | Electron Microscopy Sciences | 16365 | Electron microscopy, fixatives, final concentration is 4% in Na-cacodylate buffer, make fresh before use, shake well before to use |
| TEM | JEOL | 200CX | Electron microscopy, imaging of endocytosed vesicles and ultrastructural changes |
| CCD digital camera | AMT | XR-100 | Electron microscopy, capturing images |
| Lead citrate | Leica microsystems | 16707235 | Electron microscopy, grid staining |