B6;129S(-ROSA)26Sor/J

Stock No: 014539 | Ai39 or Ai39(RCL-eNpHR3.0/EYFP)

Targeted Mutation

AVAILABLE

PLACE ORDER

Live mice available in varying quantities. Ask Customer Service for details.
Overview

Also Known As: Ai39 or Ai39(RCL-eNpHR3.0/EYFP)

These Ai39 mice conditionally express an improved Halorhodopsin/EYFP fusion protein from the endogenous Gt(ROSA)26Sor locus. Following Cre-mediated removal of the STOP cassette, EYFP expression is observed in the cre-expressing cells. Subsequent illumination of these cells with yellow-to-red light leads to reversible photoinhibition of action potential firing/neural activity in these cells. These Ai39 mice are useful for optogenetic studies to express an inhibitory opsin that effectively silences the activity of cortical neurons.

Donating Investigator

Hongkui Zeng, Allen Institute for Brain Science

GENETIC OVERVIEW

<table>
<thead>
<tr>
<th>Genetic Background</th>
<th>Generation</th>
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<tbody>
<tr>
<td>F?N3pN1+F21</td>
<td>(2019-10-02 00:00:00)</td>
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</tbody>
</table>

Gt(ROSA)26Sor<sup>tm39(CAG-hop/EYFP)Hze</sup>

<table>
<thead>
<tr>
<th>Allele Type</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
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<tbody>
<tr>
<td>Targeted (Reporter)</td>
<td>Gt(ROSA)26Sor</td>
<td>gene trap ROSA 26, Philippe Soriano</td>
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</tbody>
</table>

RESEARCH APPLICATIONS

Research Tools

Neurobiology Research

BASE PRICE

Starting at:

$255.00 Domestic price for female
Ai39 mice heterozygous for the Rosa-CAG-LSL-eNpHR3.0-EYFP-WPRE conditional allele are viable and fertile. A \textit{loxP}-flanked STOP cassette prevents transcription of the downstream eNpHR3.0-EYFP fusion gene (see below for detailed description of eNpHR3.0-EYFP). Because the CAG promoter driven reporter construct was targeted for insertion into the Gt(ROSA)26Sor locus, eNpHR3.0-EYFP expression is determined by which tissue(s) express Cre recombinase. When bred to mice that express Cre recombinase, the resulting offspring will have the STOP cassette deleted in the cre-expressing tissues; resulting in expression of the eNpHR3.0-EYFP fusion protein. The donating investigator reports that Ai39 mice do not express eNpHR3.0-EYFP prior to introduction of Cre recombinase.

Fusion protein expression following exposure to \textit{cre} can be detected by EYFP fluorescence and mRNA (in situ hybridization) [and presumably by antibody staining (immunohistochemistry); although this was not tested by the donating investigator]. Following exposure to Cre recombinase, illuminating eNpHR3.0-expressing neurons with yellow-to-red light leads to reversible photoinhibition of action potential firing/neural activity in these cells. The donating investigator specifically reports that expression of the inhibitory opsin occurs at levels sufficient to effectively silence the activity of cortical neurons. Fusion protein expression in tissues other than brain has not yet been evaluated by the donating investigator (April 2011). The phenotype of homozygous mice has not been characterized by the donating investigator. Homozygous mice are viable and fertile.

Of note, the \textit{FRT} sites flanking the mutation allow for additional targeted replacement of the reporter sequences through \textit{Flp}-mediated recombination if so desired. Similarly, the \textit{attB}/\textit{attP}-flanked selection cassette may be removed by introduction of the site-specific bacteriophage PhiC31 integrase if so desired.

For characterization information, see images at the Allen Institute for Brain Science website (Ai39 images).

The eNpHR3.0-EYFP fusion protein is composed of the \textit{Natronomonas pharaonis}-derived halorhodopsin (NpHR) fused in-frame with an enhanced yellow fluorescent protein (EYFP). The eNpHR3.0-EYFP fusion protein has been modified to harbor both the membrane trafficking signal and the endoplasmic reticulum (ER) exporting sequence from the potassium channel \textit{Kir2.1} gene. These improvements result in optimized expression in mammalian cells by preventing ER aggregation/enhancing membrane translocation, reducing bleb formation, and enhancing inhibitory capacity. The eNpHR3.0-EYFP fusion protein allows robust photocurrents/action potential inhibition when exposed to light ranging from yellow (~589 nm) to deep red (~660 nm) and at the red/infrared border (~680 nm).

The bacterial opsins are retinal-binding proteins that combine a light-sensitive domain with an ion channel or pump; providing light-dependent ion transport, membrane potential alteration, and sensory functions to bacteria. The third-generation halorhodopsin eNpHR3.0 is an expression-optimized, yellow-to-red light-driven (~580-680 nm), inward chloride ion pump that causes hyperpolarization and prevents action potentials. For example, illumination of NpHR-expressing neurons leads to reversible photoinhibition of action potential firing/neural activity in these cells.
Genotyping Protocols
Separated PCR: Gt(Rosa)26Sor(eNpHR)
Genotyping resources and troubleshooting

Dietary Information
LabDiet® 5K52 formulation (6% fat)

Breeding Considerations
Heterozygous and homozygous mice are viable and fertile. When maintaining a live colony, heterozygous mice may be bred together, to wildtype mice from the colony or to C57BL/6J inbred mice (Stock No. 000664). Alternatively, homozygous mice may be bred together.

Additional Breeding and Husbandry Support
Mating System
Homozygote x Homozygote

Citation
When using the Ai39 or Ai39(RCL-eNpHR3.0/EYFP) mouse strain in a publication, please cite the originating article(s) and include JAX stock #014539 in your Materials and Methods section.
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