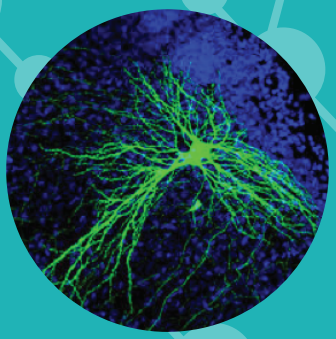
AAV Biosensors

Handbook and Product Information



Vigene Biosciences

Excellence in Gene Delivery

Legal Statement of AAV Biosensor

1. AAV Biosensors are covered under US Patents #14/350,199; #8,629,256, #14/800,814, #14/800,814, #14/941,406, #14/974,483, 14/941,406, 14/974,483, 14/452,428 and foreign equivalents and licensed from Janelia Research Campus, HHMI, Janelia, Virginia, USA.

2. The products and the reagents generated from these services shall be used as tools for research purposes, and shall exclude any human or clinical use.

3. The purchase of the AAV Biosensor Products coveys to the purchaser the limited, non-transferable right to use the products purchased and the reagents generated from Vigene Biosciences Inc. services and any related material solely for Research Purposes only, not for any Commercial Purposes.

Table of Contents

Introduction

What are biosensors?

Biosensors are genetically engineered fluorescent proteins (FP) attached to an additional protein sequence which makes them sensitive to small biomolecules or other physiological intracellular processes (e.g. Ca2+). These biosensors are introduced into cells, tissues or organisms to detect changes by fluorescence intensity or spectrum change. Many biosensors permit long-term imaging and can be engineered to specifically target cellular compartments or organelles. Additionally, biosensors permit signaling pathway exploration or allow for the measurement of a biomolecule. They do all this while principally preserving both spatial as well as temporal cellular processes.

"Optogenetic technology combines genetic targeting of specific neurons or proteins with optical technology for imaging or control of the targets within intact, living neural circuits"

Optogenetic Biosensors

Optogenetics integrates optics with genetic engineering approaches and technology. It allows for the measurement and manipulation of cells and their governing biomolecular processes. As the name suggests, the tools and technologies developed for optogenetics utilize light to detect, measure, and control molecular signals, cells, and groups of cells in order to better understand their activity plus the effects of alterations to this activity.

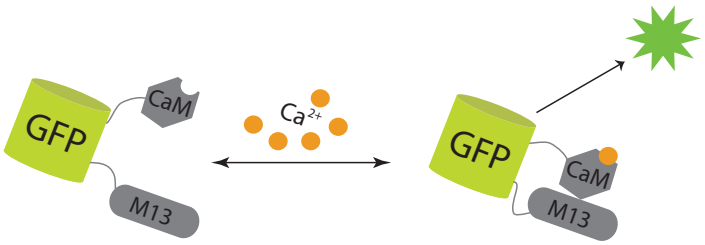
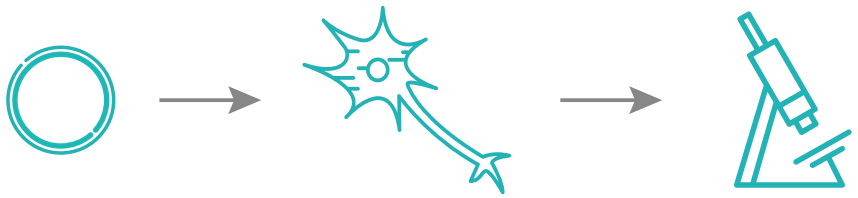


Figure: Different types of FP-based biosensor. The fusion of an FP such as GFP (green) to a specific binding domain (gray) can be used to report on the production of certain signaling molecules. Ca2+ sensors consists of a molecular switch that contains calmodulin (CaM) and M13 inserted into a circularly permuted GFP (green), in which the native N- and C-termini of GFP are linked together, and new termini are generated from within the core β-barrel structure of GFP; the addition of Ca2+ causes CaM to bind to M13, which leads to increased GFP fluorescence.

What is an AAV Biosensor?

Vigene's Biosensor AAV products come 'ready-to-use', with a choice of promoter and the ability to include the Cre inducible (FLEx-ON) expression. Additionally, we have packaged each of our AAV biosensors into the most commonly used AAV serotypes (AAV8 and AAV9). Should you require a different serotype, please contact us.



Biosensor AAV

in vivo or in vitro application

Detection, Imaging and/or quantification

Adeno-Associated Virus (AAV)

Adeno-associated virus (AAV) vectors have an advantage over other vectors mainly for the fact that they are capable of infecting a large number of cell types (proliferating and differentiating) with the same efficiency. This, as well as other characteristics, make them ideal and, in fact, commonly used in optogenetics experiments – AAV Biosensors. Other characteristics that make AAV the preferred viral vector over others, such as lentiviral vectors, is that AAV remain primarily episomal; lentiviral vectors integrate into the genome. Local chromatin structure at the site of genome integration can change the expression of transgenes. Integration events can alter expression of neighboring genes. The small insert size of channel rhodopsins, halorhodopsins, and other optogenetic genes enables them to be packaged in AAV vectors.

Genomic organization of AAV

AAV is one of the smallest single strand DNA viruses with a non-enveloped capsid, approximately 22 nm. Between 80-90% of adults are serotype-positive with AAV2, however infection has not been associated with any symptoms or disease.

The AAV genome consists of two open reading frames (ORF) Rep and Cap, which are flanked by two 145 base inverted terminal repeats (ITRs). ITRs base pair, allowing synthesis of complementary DNA strands. Rep and Cap are translated to generate multiple distinct proteins, such as Rep78, Rep68, Rep52, Rep40 - required for the AAV life cycle; as well as VP1, VP2, VP3 - capsid proteins.

When constructing an AAV transfer vector, the transgene is placed between the two ITRs, and Rep and Cap are supplied in trans. AAV additionally requires a helper plasmid, containing genes from adenovirus, namely E4, E2a and VA. These genes help mediate AAV replication. The transfer plasmid, Rep/Cap, and the helper plasmid are all transfected into HEK293 cells, which contain the adenovirus gene E1+, to produce infectious AAV particles. Rep/Cap and the adenovirus helper genes may be combined into a single plasmid; the separation of Rep and Cap enables viral pseudotyping.

AAV Serotypes

There have been 11 serotypes identified thus far, with the most characterized and commonly used being AAV2. Serotypes differ in their tropism, or the types of cells they infect. This characteristic makes AAV a very useful system for preferentially transducing specific cell types. For example, AAV serotypes 1, 2, 5, 8 and 9 have been shown to efficiently transduced cortical cells of marmoset, mouse and macaque. Whereas, AAV2 was distinct from other serotypes in neuronal tropism and small spread.

Choosing your Biosensor AAV

Vigene's current range of Biosensor AAV products (see table at the end of this booklet) come as ready-to-use AAV vectors; ready for in vivo injection.

Steps to Biosensor AAV selection:

1. Firstly, decide which biosensor is most suitable for your research question – calcium or glutamate; for GCaMP6, whether you like GCaMP s or m, f.
2. Secondly, decide whether you would like to include FLEx.
3. Thirdly, choose a promoter (universal promoter – CAG, or neuron specific-synapsin promoter).
4. Lastly, choose the AAV vector serotype (AAV1-9).

"Calcium, as an important regulator of many cellular signaling events, and calcium indicators, have found extensive use for imaging and measuring changes in Ca2+ levels associated with neural activity"

Our current available biosensors include a range of calcium and glutamate biosensors:

- CaMPARI
- GCaMP1
- GCaMP3
- GCaMP5
- GCaMP6
- jRCaMP1
- jRGECO1
- iGLuSnFR

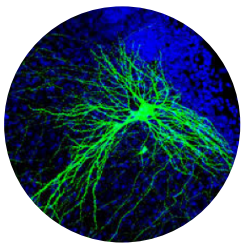
We are updating this product listing continuously. We have more exciting products to add. Available biosensor indictors are listed in the table on the opposite page. For more information on a specific product please visit our website or contact us.

Our currently available products is listed in the table at the end of this booklet.

AAV Biosensors

Calcium Indicators

CaMPARI



CaMPARI or Calcium Modulated Photoactivatable Ratiometric Integrator is a photoconvertible protein construct [1]. CaMPARI enables imaging of integrated calcium activity of large populations of cells over defined time windows. Calcium, as an important regulator of many cellular signaling events, and calcium indicators have found extensive use for imaging and measuring changes in Ca2+ levels associated with neural activity.

Typically, genetically encoded calcium indicators (GECI) exhibit rapid response to Ca2+ concentration changes. Upon binding of GECI to Ca2+ there is an induction of a change in fluorescence signal. It is this change in signal which allows for measuring of action potentials, and other receptor activation events, that trigger Ca2+ fluxes. This characteristic provides real-time information, however, it limits studies to areas within a microscope's field of view.

With the development of CaMPARI, imaging of calcium activity of large areas and within populations of cells is now possible. CaMPARI undergoes significantly faster, and permanent, green-to-red photoconversion (PC), only when calcium is present and while PC light is applied. This permanent conversion records calcium activity for all areas illuminated by PC-light. The red fluorescence intensity correlates with calcium activity.

"CaMPARI complements existing calcium indicators by enabling measurements of the total calcium activity over large areas of cells and tissues."

How it works

CaMPARI is a photoconvertible protein construct, enabling imaging of integrated calcium activity of large populations of cells over defined time windows.

CaMPARI is based on EosFP, a fluorescent protein whose emission changes from green to red upon irradiation with UV-light (~400 nm). By engineering libraries of EosFP variants, inventors were able to develop a protein that undergoes significantly faster green-to-red photoconversion (PC) only when calcium is present while PC light is applied. This permanent conversion provides the ability to record calcium activity for all areas illuminated by PC-light. The red fluorescence intensity correlates with calcium activity. CaMPARI complements existing calcium indicators by allowing total calcium activity measurement over large areas of cells and tissues.

Key advantages

Image total calcium activity during defined time windows (gated by photoconversion light).

- Not restricted to the field of view of a microscope, as during real-time calcium imaging with e.g. GCaMP.
- Enables higher-throughput calcium assays with cultured cells.
- Calcium activity imaging of across large cell populations and/or tissues.
- Labeling of "active" cells within a tissue (such as brain) during stimulus or behavior in model organisms.
- Tracing of neurons based on their calcium activity level.

CaMPARI Applications

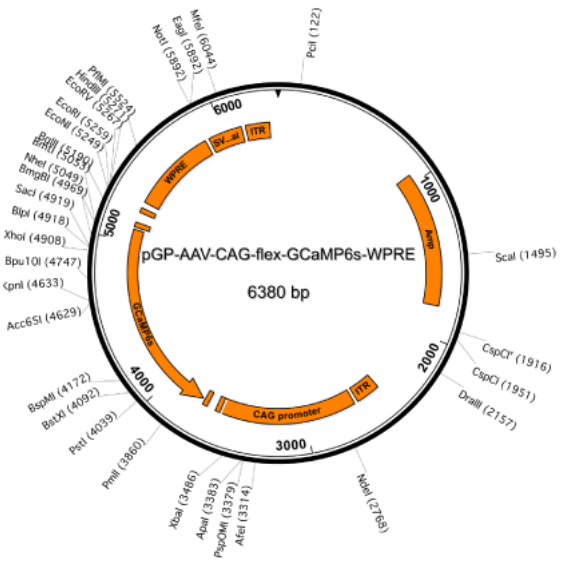
- Calcium activity imaging across large cell populations and/or tissues
- Labelling of "active" cells within a tissue (such as brain) during stimulus or behavior in model organisms
- Tracing neurons based on their calcium activity level
- Integration of subcellularity locolized calcium activity when targeted to specific subcellular locations

References

[1] Neural circuits. Labeling of active neural circuits in vivo with designed calcium integrators. Fosque BF, Sun Y, Dana H, Yang CT, Ohyama T, Tadross MR, Patel R, Zlatic M, Kim DS, Ahrens MB, Jayaraman V, Looger LL, Schreiter ER Science. 2015 Feb 13;347(6223):755-60. doi: 10.1126/science.1260922.

GCaMP Sensors: GCaMP3, GCaMP5, GCaMP6

The GCaMP [1] family of sensors are a collection of ultrasensitive, green fluorescent indicator proteins. They facilitate the measurement of synaptic calcium signals.



How it works

GCaMP is a genetically encoded calcium indicator (GECI) which was generated from a fusion of the green fluorescent protein (GFP), calmodulin, and M13, a peptide sequence from myosin light chain kinase. Upon binding of GECI to Ca2+ there is an induction of a change in fluorescence signal. It is this change in signal that allows for measurement of the action potentials, and other receptor activation events, that trigger Ca2+ fluxes.

Key advantages

- Ready for use in vivo.
- GCaMP6 are the 6th generation of GCaMP, offering improved engineering for increased signal-to-noise ratio and much faster kinetics, when compared to previous versions, specifically, GCaMP3 and GCaMP5G.
- GCaMP3 is the 3rd generation of GCaMP and reliably detects three or more action potentials in short bursts in several systems in vivo

GCaMP Advantage

- Reliable detection of single action potential responses in vivo
- Useful for studying high frequency neuronal activity
- Fastest observed kinetics

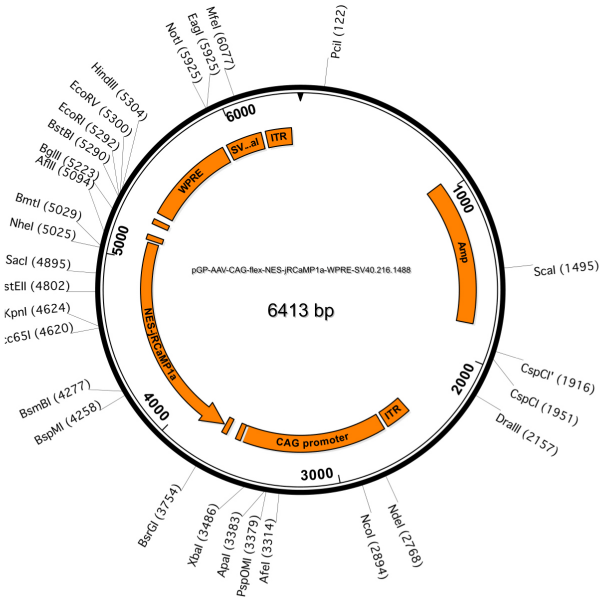
- GCaMP6 is the 6th generation of GECIs. Having new and improved engineering allows for increased signal-to-noise ratio as well as much faster kinetics, when compared to previous versions, specifically, GCaMP3 and GCaMP5G.

References

[1] Ultrasensitive fluorescent proteins for imaging neuronal activity. Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS Nature. 2013 Jul 18;499(7458):295-300. doi: 10.1038/nature12354

RCaMP Sensors: jRCaMP1a & jRCaMP1b

Belonging to the family of GECIs from fluorescent proteins other than Aquorea victoria GFP. RCaMP [1], where the 'r' refers to red ('RCaMP'), sensors are new single-wavelength GECIs. In addition to RCaMP, other family members include cyan ('CyCaMP') and yellow ('YCaMP') variants.



They facilitate the detection of neuronal action potentials, astrocyte activation and other cellular processes. Binding of Ca2+ ions to these sensors produces large fluorescence increases, detectable with generic fluorescence detection instruments. Efficacy of the RCaMP sensors has been demonstrated through in vivo imaging in flies, worms, and fish; 2-color imaging of neuron and astrocyte activity in co-culture; and integrated "read/write" optogenetics alongside channel-rhodopsin-2 (ChR2).

How it works

RCaMP is a GECI which was generated from a fusion of the red fluorescent protein (RFP), calmodulin, and M13, a peptide sequence from myosin light chain kinase. Upon the binding of GECI to Ca2+ there is an induction of a change in fluorescence signal. It is this change in signal which allows for measuring the action potentials, and other receptor activation events, that trigger Ca2+ fluxes.

Key advantages

- Provides new color channels for single-wavelength functional imaging.
- Minimal bleed through into green channel, unlike other biosensors.
- Compatible with optogenetic activation/ silencing via tools such as channelrhodopsin-2, facilitating "read/write" optogenetics.
- Tunable affinity under control of various promoters.

RCaMP Application

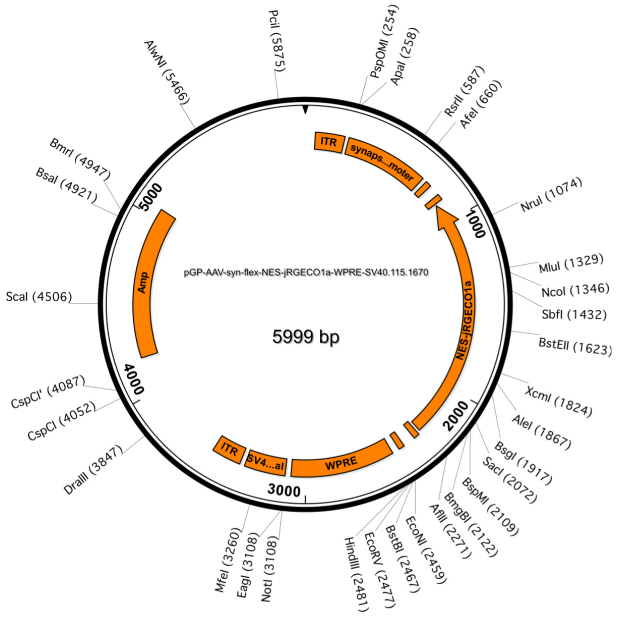
- Multifunctional imaging for drug screening.
- Imaging cellular activity in cells and organisms that already express GFP
- Less auto-fluorescence than green GECIs and dyes

References

[1] Akerboom J, Carreras Calderón N, Tian L, et al. Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. *Frontiers in Molecular Neuroscience*. 2013;6:2. doi:10.3389/fnmol.2013.00002.

GECO1: jRCEGO1a & jRCEGO1b

GECIs with red-shifted excitation and emission spectra have advantages for in vivo imaging due to reduced scattering and absorption in tissue, and a consequent reduction in phototoxicity. jRGECO1a and jRCaMP1b offer improved red GECIs based on mRuby and mApple, respectively - with sensitivity comparable to GCaMP6.



How it works

jRGECO1 is a GECI which was generated from a fusion of the mApple-based (green) fluorescent protein (FP), calmodulin, and M13, a peptide sequence from myosin light chain kinase. Upon the binding of GECI to Ca2+ there is an induction of a change in fluorescence signal. It is this change in signal which allows for measuring the action potentials, and other receptor activation events, that trigger Ca2+ fluxes.

Key advantages

- Increased red fluorescence sensitivity, comparable to GCaMP6.
- Best performer for tracking sensitive stimuli; has advantages in response speed.
- Facilitate deep-tissue imaging, dual-color imaging together with GFP-based reporters, and the use of optogenetics in combination with calcium imaging.

jRCECO1 Application

- In vivo imaging
- Deep tissue imaging
- Dual color options

References

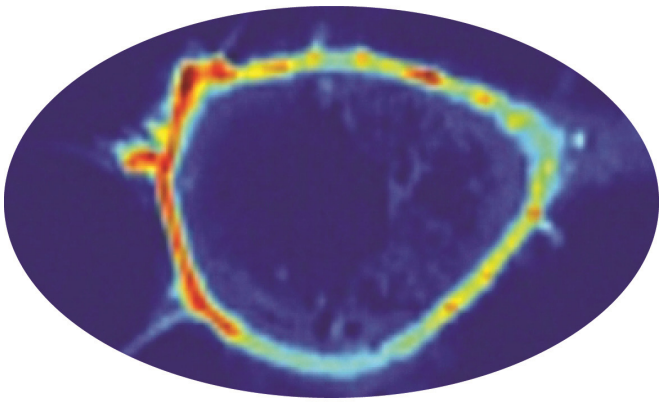
[1] Dana H, Mohar B, Sun Y, et al. Sensitive red protein calcium indicators for imaging neural activity. Häusser M, ed. eLife. 2016;5:e12727. doi:10.7554/eLife.12727.

Other Sensors

Glutamate - iGluSnFR

Glutamate, an important signaling molecule, is the major neurotransmitter in the brain, playing a critical role in nearly all aspects of normal brain function. It is released from presynapse and picked up by receptors on the postsynapse; more or less this happens on the cell surface.

How it works



Glutamate optical sensors are composed of glutamate-binding proteins coupled to fluorescent readouts. The new and improved iGluSnFR [1] has increased intensity and is constructed from E. coli GltI and circularly permutated GFP. Additionally, an optimized single wavelength glutamate sensor has been engineered in vitro for maximum fluorescence response. Given that this sensor is much brighter than existing options and has a rapid response time, it can be used for two-color imaging experiments and long-term in vivo imaging of glutamate signaling in worms, zebrafish, and mice.

The iGluSnFR construct delivers improved means to directly map excitatory synaptic activity in the brain. This construct will complement existing imaging methods for studies of neural activity and signaling events. Glutamate imaging studies in non-neuronal tissues will also benefit from the improved performance of iGluSnFR.

Key advantages

- Extremely rapid glutamate detection with high spatial resolution.
- Improved signal-to-noise ratio compared to existing fluorescent glutamate biosensors.
- Genetically-encoded, thereby can be targeted to specific cellular populations and sub-cellular locations.
- Enables direct visualization of synaptic release (as opposed to Ca2+ imaging).
- Long-term in vivo imaging of glutamate signaling in worms, zebrafish, and mice.

iGLuSnFR Application

- Neurobiology research using in vivo or in vitro models, including long term imaging studies
- Non-neuronal tissues examining glutamate signaling

References

[1] Marvin JS, Borghuis BG, Tian L, et al. An optimized fluorescent probe for visualizing glutamate neurotransmission. Nature methods. 2013;10(2):162-170. doi:10.1038/nmeth.2333.

Biosensor Products

Variant	Product Name	Cat #	Titer, Vol	Price
Calcium Sensors				
CaMPARI	pAAV-synapsin-CaMPARI	BS10-NORAAV	100ul titer at 10^13 GC/ml	$499
	pAAV-synapsin-FLEX-CaMPARI	BS10-NXRAAV	100ul titer at 10^13 GC/ml	$499
	pAAV-CAG-FLEX-CaMPARI	BS10-CXRAAV	100ul titer at 10^13 GC/ml	$499
GCaMP6s	pGP-AAV-syn-GCaMP6s-WPRE.4.641	BS1-NOSAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-syn-flex-GCaMP6s-WPRE.24.641	BS1-NXSAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-GCaMP6s-WPRE.25.641	BS1-CXSAAV	100ul titer at 10^13 GC/ml	$499
GCaMP6m	pGP-AAV-syn-GCaMP6m-WPRE.4.629	BS2-NOMAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-syn-flex-GCaMP6m-WPRE.24.629	BS2-NXMAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-GCaMP6m-WPRE.25.629	BS2-CXMAAV	100ul titer at 10^13 GC/ml	$499
GCaMP6f	pGP-AAV-syn-GCaMP6f-WPRE.4.693	BS3-NOFAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-syn-flex-GCaMP6f-WPRE.24.693	BS3-NXFAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-GCaMP6f-WPRE.25.693	BS3-CXFAAV	100ul titer at 10^13 GC/ml	$499
GCaMP3	AAV_CAG-GCaMP3	BS4-CX3AAV	100ul titer at 10^13 GC/ml	$499
Variant	Product Name	Cat #	Titer, Vol	Price
Calcium Sensors *continued*				
GCaMP3	AAV_CAG-GCaMP3	BS4-CX3AAV	100ul titer at 10^13 GC/ml	$499
GCaMP5	pRSET.GCaMP5G(7.35)	BS5-PXAAAV	100ul titer at 10^13 GC/ml	$499
jRCaMP1a	pGP-AAV-syn-NES-jRCaMP1a-WPRE.211.1488	BS6-NOAAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-syn-flex-NES-jRCaMP1a-WPRE.215.1488	BS6-NXAAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-NES-jRCaMP1a-WPRE.216.1488	BS6-CXAAAV	100ul titer at 10^13 GC/ml	$499
jRCaMP1b	pGP-AAV-syn-NES-jRCaMP1b-WPRE.211.1519	BS7-NOBAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-syn-flex-NES-jRCaMP1b-WPRE.215.1519	BS7-NXBAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-NES-jRCaMP1b-WPRE.216.1519	BS7-CXBAAV	100ul titer at 10^13 GC/ml	$499
jRGECO1a	pGP-AAV-syn-NES-jRGECO1a-WPRE.111.1670	BS8-NOAAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-syn-flex-NES-jRGECO1a-WPRE.115.1670	BS8-NXAAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-NES-jRGECO1a-WPRE.116.1670	BS8-CXAAAV	100ul titer at 10^13 GC/ml	$499
jRGECO1b	pGP-AAV-syn-NES-jRGECO1b-WPRE.111.1721	BS9-NOBAAV	100ul titer at 10^13 GC/ml	$499

Variant	Product Name	Cat #	Titer, Vol	Price
Calcium Sensors *continued*				
	pGP-AAV-syn-flex-NES-jRCaMP1b-WPRE.215.1519	BS7-NXBAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-NES-jRCaMP1b-WPRE.216.1519	BS7-CXBAAV	100ul titer at 10^13 GC/ml	$499
jRGECO1a	pGP-AAV-syn-NES-jRGECO1a-WPRE.111.1670	BS8-NOAAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-syn-flex-NES-jRGECO1a-WPRE.115.1670	BS8-NXAAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-NES-jRGECO1a-WPRE.116.1670	BS8-CXAAAV	100ul titer at 10^13 GC/ml	$499
jRGECO1b	pGP-AAV-syn-NES-jRGECO1b-WPRE.111.1721	BS9-NOBAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-syn-flex-NES-jRGECO1b-WPRE.115.1721	BS9-NXBAAV	100ul titer at 10^13 GC/ml	$499
	pGP-AAV-CAG-flex-NES-jRGECO1b-WPRE.116.1721	BS9-CXBAAV	100ul titer at 10^13 GC/ml	$499
Glutamate Sensors				
iGluSnFR	pCMV(MinDis).iGluSnFR	BS11-COGAAV	100ul titer at 10^13 GC/ml	$499

Receiving Vigene Biosensors

All AAV-biosensors come in 'ready-to-inject' and 'ready-to-transduce' format of AAV vectors. Upon receipt, please store them in -80C.

Have other questions? Please contact us. We are more than happy to answer any of your viral vector questions and needs.

Custom AAV Biosensors

Have something special in mind? Let our PhD level technical scientists help you design a custom biosensor for your research needs.

Vigene Biosciences

Ordering

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