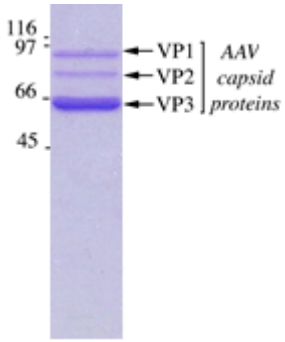
GeneDetect.com Limited

rAAV Product Information Sheet

Code	MJFF-GD1001 (GeneDetect ® rAVE™ Gene Delivery Reagent)
Vector	AAV1/2-CMV/CBA-**human-A53T-alpha-synuclein**-WPRE-BGH-polyA
Vector description	AAV1/2 Expression Vector. The CMV/CBA promoter consists of the chicken β-actin promoter hybridized with the CMV immediate early enhancer sequence and is highly efficient in most tissue types. The Woodchuck post-transcriptional regulatory element (WPRE) and the presence of a bovine growth hormone (BGH) polyadenylation sequence ensures high transcription following transduction.
Lot Number	36572
Quantity	0.2 mL
Titer	5.1 x 10^{12} genomic particles/ml
Purity	Affinity purified against immobilized heparan sulfate proteoglycan. Concentrated by modified Iodixanol/cation exchange/Q-Sepharose.
Presentation	Liquid in phosphate buffered saline (PBS) containing 1mM $MgCl_2$
Storage & stability	Upon receipt, briefly spin contents of vial to collect sample, aliquot on ice as necessary and store: 4°C for short term (<1 month), -20 °C or -80°C for long term. Avoid repeated freeze-thaw cycles.
Quality control	10μl was analyzed by SDS-PAGE to verify purity.



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Handling

Always wear laboratory gloves, protective glasses and a suitable protective laboratory coat when using rAVE™ reagents. Recent NIH guidelines state that "adeno-associated virus (AAV) types 1 through 4, and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus" can in most cases be handled at biosafety level 1 (BL1). You should follow the guidelines set by your Institutional biosafety committee for the handling of adeno-associated virus.

Disposal

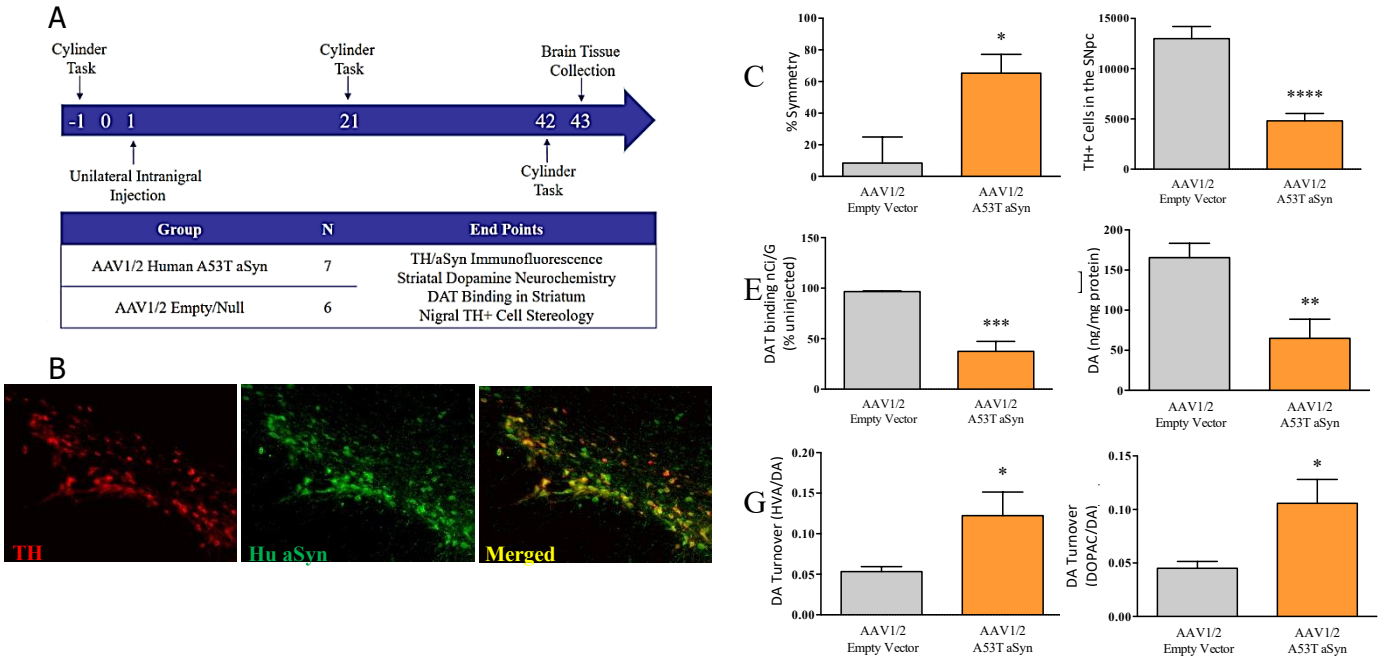
rAVE™ reagents are susceptible to 5% phenol, 10% bleach, 10% Wescodyne or Virkon. We recommend using a fresh solution of 10% bleach for 30 minutes for decontamination.

Applications

For *in vitro* applications, mix 2µl rAVE™ sample with 200µl pre-warmed culture media and apply per well to cells of 60 - 80% confluency (24well plate). Allow at least three days for viral integration and gene expression before analysis. For *in vivo* applications, dose should be determined by end user.

Refer to www.GeneDetect.com for a selection of protocols.

MJFF In Vivo Testing



Nigrostriatal degeneration induced by unilateral intranigral injection of AAV1/2 A53T aSyn in Sprague Dawley rat. A) Overview of the experimental design. Timeline of behavioral analyses and brain tissue collection relative to unilateral intranigral injection of the AAV1/2 Human A53T aSyn (n=7) or AAV1/2 Empty Vector (n=6) and summary of endpoint analyses. A dose of 2.58 x 10^{12} gp/mL was used for each virus following the injection procedure described in Koprich et al, 2011. B) Immunofluorescent staining for colocalization of human aSyn and TH expression in the ipsilateral SNpc 43 DPI of the AAV1/2 A53T aSyn vector. At 43 DPI, the majority of TH-immunoreactive neurons of the SNpc display high levels of human aSyn expression, as well as some TH-negative cells within the boundary of the SNpc. C) Cylinder task at 43 DPI for motor deficits induced by unilateral intranigral injection of viral vector. AAV1/2 A53T aSyn-injected rats displayed significantly increased forelimb asymmetry as compared to AAV1/2 empty vector controls. D) Stereological cell counts for immunolabeled TH+ cells in the SNpc at 43 DPI. AAV1/2 A53T aSyn resulted in a significant reduction of TH+

cells in the SNpc as compared to the empty vector control. E) Autoradiography for dopamine transporter at 43 DPI in AAV1/2 A53T aSyn and empty vector controls. AAV1/2 A53T aSyn injection resulted in a significant ~50% reduction in dopamine transporter binding as compared to the uninjected hemisphere whereas the empty vector control had no impact on dopamine transporter binding. F-H) Striatal dopamine neurochemistry at 43 DPI as assessed by LC/MS. F) Striatal dopamine levels are significantly reduced in the AAV1/2 A53T aSyn versus the AAV1/2 empty vector control group. This reduction corresponds to increased readouts of dopamine turnover as analyzed by HVA/DA levels (G) and DOPAC/DA levels (H). Bars represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 by t-test. Abbreviations: aSyn, alpha-synuclein; DPI, days post-injection; TH, tyrosine hydroxylase; DAT, dopamine transporter; TH+, tyrosine hydroxylase-positive; SNpc, substantia nigra pars compacta; DA, dopamine; LC/MS, liquid chromatography mass spectrometry; HVA, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; SEM, standard error of the mean.

References

- Koprich *et al.* (2010). Expression of human A53T alpha-synuclein in the rat substantia nigra using a novel AAV1/2 vector produces a rapidly evolving pathology with protein aggregation, dystrophic neurite architecture, and nigrostriatal degeneration with potential to model the pathology of Parkinson's disease. *Molecular Neurodegeneration,* 5:43.
- Koprich *et al.* (2011). Progressive neurodegeneration or endogenous compensation in an animal model of Parkinson's disease produced by decreasing doses of alpha-synuclein. *PLoS ONE,* 6(3): e17698.
- He *et al.* (2015). Treatment with trehalose prevents behavioral and neurochemical deficits produced in an AAV alpha-synuclein rat model of Parkinson's Disease. *Molecular Neurobiology,* 53(4): 2258-2268.
- Koprich *et al.* (2016). Towards a non-human primate model of alpha-synucleinopathy for development of therapeutics for Parkinson's disease: optimization of AAV1/2 delivery parameters to drive sustained expression of alpha-synuclein and dopaminergic degeneration in Macaque. *PLoS ONE*, 11(11): e0167235.
- Ip *et al.* (2017). AAV1/2-induced overexpression of A53T-alpha-synuclein in the substantia nigra results in degeneration of the nigrostriatal system with Lewy-like pathology and motor impairment: a new mouse model for Parkinson's disease. *Acta Neuropathologica Communications,* 5:11.
- Musacchio *et al.* (2017). Subthalamic nucleus deep brain stimulation is neuroprotective in the A53T α-synuclein Parkinson's disease rat model. *Annals of Neurology,* 81(6): 825-836.
- Gleave *et al.* (2017). Sirtuin 3 rescues neurons through the stabilisation of mitochondrial biogenetics in the virally-expressing mutant α-synuclein rat model of parkinsonism. *Neurobiology of Disease,* 106: 133-146.
- Schneider *et al.* (2019). GM1 Ganglioside modifies alpha-synuclein toxicity and is neuroprotective in a rat alpha-synuclein model of Parkinson's disease. *Sci Rep,* 9(1): 8362.
- Badr *et al.* (2022). Expansion of regulatory T cells by CD28 superagonistic antibodies attenuates neurodegeneration in A53T-alpha-synuclein Parkinson's disease mice. *J Neuroinflammation,* 19(1): 319.
- Karikari *et al.* (2022). Neurodegeneration by alpha-synuclein-specific T cells in AAV-A53T-alpha-synuclein Parkinson's disease mice. *Brain Behav Immun,* 101: 194-210.
- Musacchio *et al.* (2022). Temporal, spatial and molecular pattern of dopaminergic neurodegeneration in the AAV-A53T alpha-synuclein rat model of Parkinson's disease. *Behav Brain Res,* 432: 113968.
- Hofman *et al.* (2025). Low beta predicts motor output and cell degeneration in the A53T Parkinson's disease rat model. *Brain,* awaf063.

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