

Code	MJFF-GD1004 (GeneDetect <sup>®</sup> rAVE <sup>™</sup> Gene Delivery Reagent)
Vector	AAV1/2-CMV/CBA- <b>Null/Empty</b> -WPRE-BGH-polyA
Vector description	AAV1/2 Expression Vector. The CMV/CBA promoter consists of the chicken $\beta$ -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	36573
Quantity	0.2 mL
Titer	5.1 x 10 <sup>12</sup> 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 <sub>2</sub>
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	$10\mu$ l was analyzed by SDS-PAGE to verify purity.



Note: GeneDetect  $^{\circledast}$  and rAVE^{TM} are trademarks of GeneDetect.com Limited.

Handling	Always wear laboratory gloves, protective glasses and a suitable protective laboratory coat when using rAVE <sup>™</sup> 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 <sup>™</sup> 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 <i>in vitro</i> applications, mix $2\mu$ l rAVE <sup>TM</sup> sample with $200\mu$ l 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 <i>in vivo</i> 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<sup>12</sup> 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  $\pm$  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

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