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Certificate of Origin and Health

and Non-Infectious Status

AMV RT is derived from the BAI/A strain of Avian Myeloblastosis Virus. Life Sciences, Inc., in St. Petersburg, FL, has maintained the virus here with minimal passage. AMV characteristically causes viral proliferation and a myeloblastic leukemia in chicks.

A study by Baluda, Moscovici and Goetz¹ surveyed the ability of AMV to grow in and cause proliferation of tissue cultures of yolk sac, liver and spleen of three orders of birds. Virus multiplication was limited to birds of the order Galliformes (chickens, turkeys and quail) and only the chicken and quail cells showed any evidence of the virus-induced proliferation. No viral multiplication or oncogenic conversion was observed in mammalian cell cultures from mouse, rat, hamster, guinea pig or human fetus. *The above data serves as evidence for the very specific target cell requirements for AMV infection and cell conversion, and argues strongly for the inability of this virus to infect humans. We do not know of any case of human infection at Life Sciences, Inc., or anywhere else.*

Analysis of dehydrated avian Myeloblastosis virus has been shown to consist of 35% lipid, 2% RNA, and about 60% protein and a small amount of carbohydrate². It is ether sensitive, and completely disrupted by lipid solvents such as chloroform-methanol 2:1. Electron microscopic studies performed by Bonar, Heine and Beard showed disruption of the two lipid-containing membranes surrounding the inner nucleoprotein core by Tween 80, a non-ionic detergent. *We do not believe that infectious virus survives after the process of isolation of AMV reverse transcriptase enzyme.* The isolation process is characterized by:

- 1. Initial disruptive lysis in 2% non-ionic detergent in which it is homogenized in high salt solution to accomplish solubilization of the viral proteins, and release of AMV RT activity.
- 2. The purification process involves the constant presence of 0.2% Triton X100 for a period of days, which would accomplish the lysis of residual virus, if any.
- 3. Two cycles of adsorption, washing and selective elution of AMV RT from ion-exchange resins effectively separate AMV RT from other viral components.

4. A final gel-filtration step efficiently removes virus and non-RT materials from the RT preparation, resulting in enzyme that is >95% pure.

The cargo designation for AMV RT according to Schedule B No. 3507.90.000-3 is "Medical Enzyme" with no hazardous designation assigned. In addition, IATA (International Air Transportation Association) does not require us to ship RT as "Dangerous/Infectious Goods."

In view of the demonstrated biological specificity of AMV for chicks, the need for large doses of virus to bring about the disease (10¹⁰ virus particles per chick), and the stringency of the RT purification procedure, RT preparations would be highly unlikely to pose a threat of infection to humans or other animals.

Catalog No.:	BRT 43ML
Product Name:	Reverse Transcriptase of Avian Myeloblastosis Virus (AMV RT)
Source:	Avian Myeloblastosis Virus
Classification:	Avian Oncornavirus, lipid enveloped, RNA core
Animal Host:	Chickens
Tissue used:	Blood

Country of Origin of Biological components: U. S .A. The product contains no components that originate in a third country.

Chicken production facility inspected by the U.S.D A.? YesIf yes, indicate U.S.D A. site number: NPIP # 57-317Age of chicks at time of use: 1 dayIncubation period: 8-10 daysCountry of Manufacture of enzyme product: U. S. A.

Type of Purification: Purification of the virus by differential centrifugation and sucrose density gradient fractionation, followed sequentially by detergent lysis and isolation of the enzyme (RT) by a three-step chromatographic process.

The avian myeloblastosis virus has not been reported to be pathogenic for humans. However, the purification scheme employed in the recovery of the enzyme involves a detergent lysis step that has been shown to cause the solubilization and resultant inactivation of the virus. This procedure would also result in the solubilization and activation of Newcastle disease virus and avian influenza virus, all of which are RNA viruses with ether-sensitive lipid envelopes that are readily lysed by detergent.

References:

¹ "Specificity of the Inductive Effect of Avian Myeloblastosis Virus," Baluda M.A., C. Moscovici, and I. E. Goetz. pp. 449-458

² "Structure of BAI Strain A (Myeloblastosis) Avian Tumor Virus". Bonar R.A., Ursula Heine, and J. W. Beard. *National Cancer Institute Monograph* No. 17 (1964) pp 589-614