



Standard Practice for Measurement of the Biological Activity of Ricin¹

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INTRODUCTION

Ricin is a member of the protein toxins that cause their physiological effect by inactivation of ribosomes. Ricin is a member of the class 2 ribosome inactivating proteins (1).² Other members of this class of toxins include the proteins abrin and Shiga toxin.

Ricin consists of two chains, the A-chain that is responsible for the N-glycosidase enzymatic activity and the B-chain that is needed for cell binding and intra-cellular processing. Ricin is a heterogeneous protein with molecular weights ranging from approximately 62 to 64 kilodaltons (kDa) (2). Both chains are glycosylated and of similar size (approximately 32 kDa). There are several genes encoding putative ricin and ricin-like proteins in the genome of *R. communis* (3) resulting in differences in the amino acid sequence of the subunits. The differences in amino acid sequence and glycosylation both contribute to the heterogeneity of ricin.

1. Scope

1.1 This guide is intended for the manufacturers and users of ricin reference material. Ricin reference materials are well-characterized materials that can be used to test detection devices and calibrate laboratory measurements. It is anticipated that ricin reference materials will be characterized by biochemical methods in addition to the measurement of biological activity.

1.2 This practice details the measurement of ricin biological activity using a cell-free translation (CFT) assay (4).

1.3 The CFT assay has been developed for use in any biotechnology laboratory where determination or confirmation of ricin biological activity is required.

1.4 The CFT assay has been validated by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) VP-016 Validation of Cell-Free Translation Assay for the Detection of Ricin Toxin Biological Activities in compliance (5) with Good Laboratory Practices (GLP) Regulations of the Food and Drug Administration (21 CFR Part 58). Strict adherence to the protocol is necessary for validity of the test results.

1.5 **Appendix X1** and **Appendix X2** also provide guidance for the measurement of the biological activity of ricin using cell-based assays and the use of synthetic enzyme substrates.

1.6 Ricin is a category 2 select agent and acquisition of the ricin standard must adhere to the Center for Disease Control (CDC) regulations. Ricin is listed on the select agent list (42 CFR Part 72).³ The possession, transfer, and use of ricin are restricted under the Public Health Security Preparedness Act (CRS Report RL31263 Public Health Security and Bioterrorism Preparedness and Response Act (P.L. 107-188): Provision and Changes to Preexisting law). Access to stores of ricin is limited (USA Patriot Act, P.L. 107-56). Ricin is also a prohibited substance under the Biological Weapons Convention and the Chemical Weapons Convention (CRS Report RL31559 Proliferation Control Regimes: Background and Status).

1.7 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.8 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Ricin is an extremely dangerous toxin. See Section 9 for specific hazards information.

¹ This practice is under the jurisdiction of ASTM Committee E54 on Homeland Security Applications and is the direct responsibility of Subcommittee E54.01 on CBRNE Sensors and Detectors.

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² The boldface numbers in parenthesis refer to the list of references at the end of this standard.

³ Available at <http://www.bt.cdc.gov/Agent/agentlist.asp>.