Centre for AIDS Reagents

Data Sheet

REPOSITORY REFERENCE: **ARP6014**

NAME: HIV-1 Rev (Wild Type)

PRODUCTION: The *rev* cDNA from pCV-1 (NIH Cat #303) was amplified using PCR and cloned into the expression vector pOTS *Nco*1 (SmithKline Beecham). This plasmid was transformed into *E. coli* N5151 and thermally induced. The crude Rev was re-suspended in Buffer A (50 mM Tris-HCl, pH 8.0, 150 mM NaCl), then ammonium sulfate precipitated, filtered, and the filtrate put over an FPLC column equilibrated with 60% buffer A and 40% buffer B (50 mM Tris-HCl, pH 8.0, 1.5 M NaCl). The protein was eluted using a linear gradient of 40-100% buffer B in buffer A.

Diluting the protein into a lower salt buffer will cause it to aggregate and precipitate. This protein is functional when introduced into mammalian cells.

BUFFER: 50 mM Tris-HCl, pH 8.0 and 0.3 M NaCl

RECOMMENDED STORAGE: -70°C

PRESENTATION: 75µg at 0.55 mg/ml in 0.5 M NaCl, 50 mM Tris HCl, pH 8.0

SOURCE: Mr. Michael J. Orsini, Dr. David Rekosh, and Dr. Marie Louise Hammarskjöld. (Courtesy of the NIH)

REFERENCES:

Orsini MJ, Thakur AN, Andrews WW, Hammarskjold ML, Rekosh D. Expression and purification of the HIV type 1 Rev protein produced in Escherichia coli and its use in the generation of monoclonal antibodies. AIDS Res Hum Retroviruses 11:945-953, 1995.

NOTE: Corporate requests should be directed in writing to Dr. David Rekosh or Dr. Marie-Louise Hammarskjöld, University of Virginia, Department of Microbiology, Jordan Hall, 1300 Jefferson Park Avenue, Charlottesville, VA 22908.

Limited to five aliquots per laboratory.

ACKNOWLEDGEMENTS: Publications should acknowledge the donor of the reagent and the Programme EVA Centre for AIDS Reagents. Suggested wording can be found on our website in the "Acknowledgement" section at:-

www.nibsc.ac.uk/spotlight/centre_for_aids_reagents.aspx

Please also ensure that you send us a copy of any papers resulting from work using reagents acquired through CFAR, this can be by e-mail or printed copy.