

# BioReliance

## Biologics Safety Testing Services

### PERT-Ultra: A New Member of the BioReliance PERT Family

#### Introduction

For gene therapy products and vaccines produced in continuous cell lines where downstream processing is minimal, it is essential that the products and cells do not contain replication competent retroviruses. BioReliance offers a range of Fluorescent Product Enhanced Reverse Transcriptase (PERT) assays designed to detect the presence of retroviral reverse transcriptase (RT) to provide you with the best possible assay for your product. Our newest addition to this range is the PERT-Ultra assay for detection and quantification of reverse transcriptase activity by ultracentrifugation and Quantitative PERT.

#### Factors to Consider When Choosing Your Assay

##### Test Articles Known to Contain Polymerase Activity

The test sample itself can affect sensitivity and assay performance and produce results without clear information on product safety. Thus, it is important to select an assay that is suitable for a sample where high DNA or RNA polymerase activity may obscure the result. Samples which are most likely to contain cellular DNA polymerase activity include cell lysates, virus seeds, and unpurified bulk harvests. Although the chance of obtaining amplification signals in these sample types is relatively low, the risk of low level false positive signals cannot be discounted. This risk can be minimised via the removal of free polymerases by ultracentrifugation of the sample.

##### Inhibitory Agents

Samples containing certain supplements, e.g. anti-foaming agents or high serum concentrations, exhibit strong inhibitory activity on the PERT assay in the absence of ultracentrifugation. The client again risks obtaining results without clear indication of retroviral content.

#### The PERT-Ultra Solution

BioReliance has experience working with a wide variety of samples and has developed assays to meet your specific testing requirements (see Table 1). The **PERT-Ultra** assay has been developed with these requirements in mind, combining sample concentration and purification by ultracentrifugation with an extremely high sensitivity of detection by one-step RT-PCR, making **PERT-Ultra** suitable for an extremely wide range of sample types. (see Table 2)

Table 1: The BioReliance PERT Family of Assays

	PERT Assay Number		
	107323.BUK	107324.BUK	PERT-Ultra (107325.BUK)
<b>Type</b>	Quantitative	Qualitative	Quantitative
<b>Sample preparation</b>	Clarification only	Clarification only	Clarification and ultracentrifugation
<b>Detection limit*</b>	10 <sup>-7</sup> U RT enzyme	10 <sup>-7</sup> U RT enzyme	10 <sup>-7</sup> U RT enzyme 10 <sup>2</sup> IU MLV
<b>Quantitation limit*</b>	10 <sup>-7</sup> U RT enzyme	10 <sup>-7</sup> U RT enzyme	10 <sup>-7</sup> U RT enzyme 10 <sup>2</sup> IU MLV

\* per PCR reaction

New assay designed for best possible detection of retroviral reverse transcriptase (RT)

BioReliance now has a range of assays to suit every sample type

BioReliance's PERT assay is 10<sup>6</sup>-fold more sensitive than conventional RT assays

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### PERT-Ultra: A New Member of the BioReliance PERT Family, continued

Table 2: Choose a member of the BioReliance PERT family based on your sample.

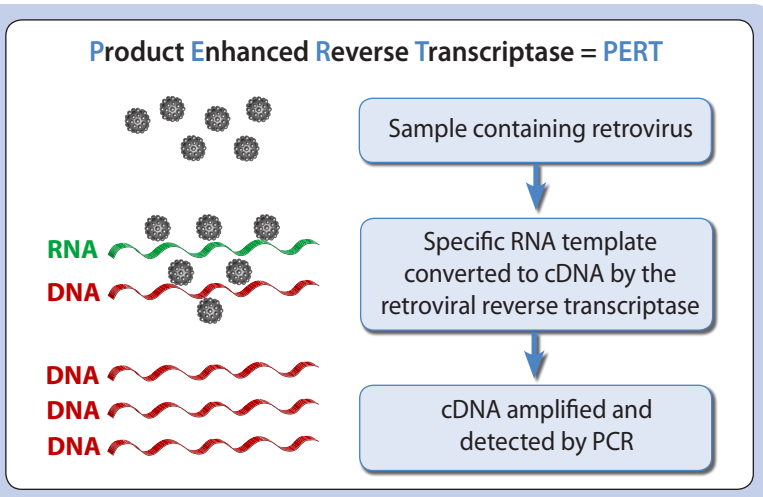
	107323.BUK	107324.BUK	PERT-Ultra (107325.BUK)
High levels of polymerase	X	X	✓
Inhibitory factors present	X	X	✓
Very high throughput	✓	✓	X
GLP compliant	✓	✓	✓
GMP compliant	✓	✓	✓
Quantitation required	✓	X	✓

### Ordering Information

Assay Number	Assay Description	Regulatory Compliance	Sample Requirements
107323.BUK 107323GMP.BUK	Detection and Quantification of Reverse Transcriptase activity by Quantitative PERT Assay	ICH Q5A, 1993 PTC, 1997 PTC	0.5 ml
107324.BUK 107324GMP.BUK	Detection of Reverse Transcriptase activity by PERT Assay	ICH Q5A, 1993 PTC, 1997 PTC	0.5 ml
107325.BUK 107325GMP.BUK	Detection and Quantification of Reverse Transcriptase activity by ultracentrifugation and Quantitative PERT Assay	ICH Q5A, 1993 PTC, 1997 PTC	2 ml

### PERT Assay Principle

The BioReliance PERT assay is an extremely sensitive assay for the detection of reverse transcriptase (RT) activity and has been reported to be up to 10<sup>6</sup>-fold more sensitive than conventional RT assays<sup>†</sup>. The assay is an RT dependent polymerase chain reaction (PCR) and therefore combines the broad specificity of conventional RT assays with the high sensitivity of PCR. Like conventional RT assays, it is used to detect RT activity packaged into extracellular retrovirus particles. The assay involves converting an RNA template to cDNA and amplifying the cDNA using product specific primers. Since no exogenous RT activity is added, cDNA will only be generated if the sample itself contains RT activity. If no RT activity is present, no product will be detected. This study uses TaqMan® technology with the real time detection of specific PERT reaction product.



<sup>†</sup> Arnold, B.A., Hepler, R.W., Keller, P.M., 1998. One step fluorescent probe product enhanced reverse transcriptase assay. *Biotechniques* 25, 98–106.

Heneine, W., Yamamoto, S., Switzer, W.M., Spira, T.J., Folks, T.M., 1995. Detection of reverse transcriptase by a highly sensitive assay in sera from persons infected with human immunodeficiency virus type 1. *J. Infect. Dis.* 171, 1210–1216.

Pyra, H., Böni, J., Schüpbach, J., 1994. Ultrasensitive retrovirus detection by a reverse transcriptase assay based on product enhancement. *Proc. Nat. Acad. Sci. USA* 91, 1544–1548.

Robertson, J.S., Nicolson, C., Riley, A.M., Bentley, M., Dunn, G., Corcoran, T., Schild, G.C., Minor, P., 1997. Assessing the significance of reverse transcriptase activity in chick cell-derived vaccines. *Biologicals* 4, 403–414.

Silver, J., Maudru, T., Fujita, K., Repaske, R., 1993. A RT PCR assay for the enzyme activity of reverse transcriptase capable of detecting single virions. *Nucleic Acids Res.* 21, 3593–3594.

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