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## PRODUCT DATA SHEET

### RECOMBINANT RABBIT TISSUE FACTOR (rRTF)

#### **Product Codes**

41055-0  
41055-1  
41055-2  
41055-3

#### **Pack size:**

100 µg  
5 mg  
1 mg  
50 mg

#### **Description:**

Recombinant Rabbit Tissue Factor (rRTF) is supplied as a ~45 kD molecule. This apo-protein contains extra cellular and transmembrane domains of the tissue factor, plus an amino terminal leader sequence consisting of several domains which aid in expression and purification.

#### **Physical State:**

Clear, colorless liquid; rRTF is supplied in 0.01 M CHAPS, 0.5 M NaCl, 0.25 M imidazole, and 0.02 M Tris-HCL, pH 7.9. This protein is shipped without added preservatives.

#### **Testing:**

Prothrombin Time: Citrol Level 1: 9.2 seconds ± 1.0 second  
Estimated Protein Purity via SDS-PAGE: 80% ± 10%  
Protein Concentration: 240 ± 15 µg/mL

#### **Packaging, shipping/storage:**

##### **Packaging**

Polypropylene, leakproof tube

##### **Storage Temperature**

2 – 8 °C

##### **Shipping Conditions**

Wet ice or cool packs

##### **Expiration**

Product quality is guaranteed to meet Pel-Freez Biologicals' specifications for 1 year from the date of receipt by the customer as long as the product is stored in accordance with the indicated storage conditions.

#### **Application Notes:**

Human tissue factor has been cloned and expressed in both E. coli and Baculovirus expression systems and successfully configured into very sensitive thromboplastin reagents. However, these reagents have shown to be sensitive to Proteins Induced by Vitamin K Antagonism or Absence (PIVKAs) in patient clinical samples which can detrimentally affect laboratory test results. Thus a project was undertaken to clone and express rabbit tissue factor to overcome the PIVKA sensitivity and create a PT reagent raw material which builds upon the vast experience of using rabbit derived thromboplastins in the clinical laboratory setting. Using the published cDNA gene sequence for rabbit brain tissue factor, the gene was



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isolated and inserted into a prokaryotic expression system. The protein was expressed as a fusion protein to increase both the solubility and the biological activity of the rRTF molecule. The result is a protein with a molecular weight of approximately 45 kD including the 12 kD fusion partner. After lipidation with synthetic phospholipids or phospholipids purified from rabbit brain, the rRTF has functional prothrombin activity in a normal prothrombin clotting assay. Cleavage of the fusion protein did not increase the activity of the molecule. Thus, a thromboplastin reagent made with rRTF may be useful as a raw material for a clinical prothrombin reagent.

**References:**

Denson, K.W.E, et al "Comparative Studies of Rabbit and Human Recombinant Tissue Factor Reagents", *Thromb Res* 1999;94: 255-61.

*Methods in Enzymology* Vol 222, 1993, pages 20-224: Proteolytic Enzymes in Coagulation, Fibrinolysis, and Complement Activation, Part A: Mammalian Blood Coagulation Factors and Inhibitors.

Laduca, F., Lee, T., El Rouby, S., Zucker M., Cohen, M.; International Technidyne Corporation, USA; ITC, USA; Newark-Beth Israel, USA: A novel recombinant tissue factor assay for measuring the anti-Xa effect of Low-Molecular-Weight Heparin (LMWH): Implications for interventional coronary procedures; Abstract # P1895, *Journal of Thrombosis and Haemostasis*, Volume 1, Supplement 1, July 12-18 2003.