



## **HEPARIN RESISTANT RECALCIFYING SOLUTION** **(HRRS)**

**Product Code X9107-HK (10 x 5ml)**

### **INTENDED PURPOSE**

This Heparin Resistant Recalcifying Solution (HRRS) is intended for use in aPTT's and surface activated clotting tests (SACT-II) such as the kaolin clotting test (KCT). Test plasmas giving prolonged aPTT, SACT-II or KCT results with regular calcium chloride may contain heparin or heparin-like substances which may be responsible for the test prolongation. Such plasmas will usually give shorter results when the aPTT, SACT or KCT is repeated using the heparin resistant recalcifying solution, instead of the regular M/40 calcium chloride. The degree of shortening can be used as an indicator of heparin activity.

### **TEST PRINCIPLES**

Polybrene and other positively charged polymers have long been used to neutralize heparin in plasmas. However positively charged polymers bind to anionic contact activating agents used in 2 stage clotting tests such as the aPTTs and prolong such tests in an unpredictable manner. Thus heparin neutralization with polybrene added to plasma or aPTT reagents is unreliable. However polybrene added to calcium solutions does not affect contact activation but does neutralize heparin if it is present in a test plasma sample. Thus aPTT results which tend to be shorter when heparin resistant recalcifying solution is used rather than plain calcium chloride indicate that heparin or a heparin-like agent may be present in the test plasma. By using HRRS it can be shown that heparin does not interfere with contact activation.

### **PRESENTATION**

10 x 5ml Heparin Resistant Recalcifying Solution (HRRS) containing 0.025M calcium salts with polybrene, with preservatives, blue marker dye, and buffers. Solution is ready to use.

### **STORAGE AND STABILITY**

Store unopened vials in the refrigerator (2°C-8°C). Heparin Resistant Recalcifying Solution (HRRS) is stable until the expiration date on the labels.

### **WARNINGS AND PRECAUTIONS**

Heparin resistant recalcifying solution is for "in vitro" diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.

Results obtained with different aPTT reagents may vary slightly with this product as shown below and thus should be interpreted with caution. In particular low molecular weight (LMW) heparins and plasmas with the additional abnormalities may be corrected to varying degrees.

aPTT Reagent						
	Actin FS		Platelin LS		Intrinsic LR	
TEST PLASMA	Regular CaCl <sub>2</sub>	HRRS	Regular CaCl <sub>2</sub>	HRRS	Regular CaCl <sub>2</sub>	HRRS
Normal QCP	32.7s	34.7s	34.7s	34.4s	32.5s	30.5s
+0.2u/ml Hep	48.9s	34.9s	52.7s	37.2s	62.7s	32.5s
+0.3u/ml	65.7s	36.4s	68.2s	39.7s	94.2s	32.7s
+0.4u/ml	88.7s	36.7s	86.2s	40.7s	134s	33.5s
+0.4u/ml LMW Heparin	49.9s	37.9s	57.7s	44.4s	55.5s	34.7s
Abnormal QC plasma (HBM)	45.2s	43.4s	47.7s	43.9s	44.2s	38.7s
AP+0.2u/ml heparin	102s	44.4	102s	53.9s	116s	40.5s
AP+0.2u/ml Clexane	62.7s	44.9	96.2s	53.2s	95.5s	41.0s

### SPECIMEN PREPARATION

**Specimen:** Plasma obtained from whole blood venous collection and 3.2% sodium citrate as an anticoagulant is the specimen of choice.

### Specimen collection and preparation:

1. Obtain venous blood sample by venipuncture.
2. Immediately evacuate into a suitable test tube containing suitable anti-coagulant (Na<sub>3</sub> citrate 0.109M) and mix well by inversion.
3. Centrifuge specimen at 1500g for 20 minutes.
4. Remove plasma from tubes within 60 minutes.
5. Test plasma sample within 4 hours for optimum results or store frozen at -20°C for up to 2 weeks. Avoid freeze thawing technique. (NCCLS Standard H21-A2<sup>1</sup>)

### TEST PROCEDURE

1. To a coagulation cuvette, add 0.1ml of plasma and 0.1ml of either aPTT, KCT or SACT reagent
2. Incubate plasma and reagent between 180 and 300 seconds. (Ensure consistent activation times)
3. Add standard Calcium Chloride 0.025M (pre-warmed to 37°C) while simultaneously starting a timer. Record clotting times in seconds.

4. If a sample has a prolonged aPTT/SACT/KCT time and to check for heparin in the test sample, repeat the test using the same volumes and conditions with Heparin Resistant Recalcifying Solution.

## **REFERENCES**

1. National committee for Clinical Laboratory Standards, Collection, Transport, and processing of Blood specimens for coagulation testing and general performance of coagulation assays: Approved guideline-third edition NCCLS publication H21-A3, Villanova, PA, 1998