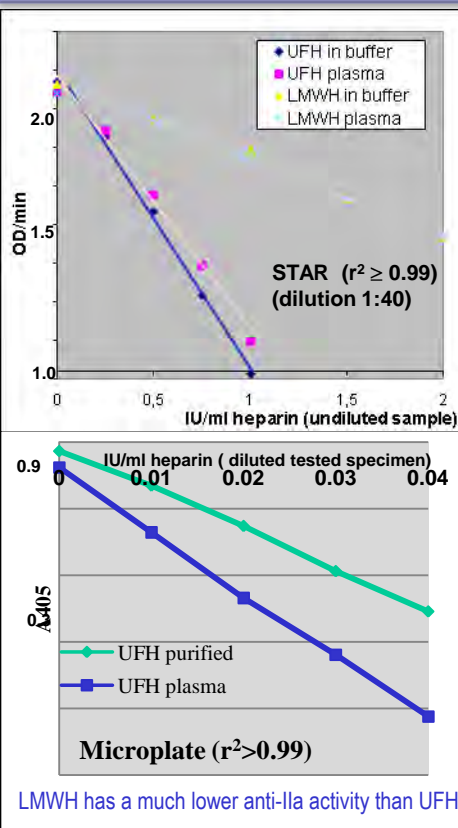


Intended use and applications

☑**RUO:** Determination of heparin anti-IIa activity in human citrated plasma or purified solutions, using a 2 stages chromogenic method, manual or automated, in compliance with USP/FDA guidelines and adjustable for EP.

Heparin + AT → [AT Hep.]
[AT Hep.] + [IIa (excess)] → [FIIa-AT-Hep.] + [residual FIIa]
[FIIa (residual)] + IIa-Subs. → Peptide + pNA

Calibration curves (STAR, microplate)



Related products

1. Biophen Heparin (#A221003/A221006)
2. Biophen Heparin anti-Xa (2 stages) (#A221010)
3. Biophen Heparin anti-IIa (kinetics) (#A221020)
4. Biophen Heparin Calibrators and controls for UFH or LMWH

Characteristics and advantages

- **User friendly protocol in compliance with USP recommendations; and adjustable for use with the EP protocol.**
- **Simple and rapid:** lyophilised and ready to use reagents; total assay time < 3 min.
- Easy to use on major **coagulation analyzers, microplate or with basic equipment** (~140 (STAR) to 250 (microplate) tests / kit).
- **Associated plasma calibrators and controls** validated against the International Standard for UFH and LMWH (NIBSC).
- Dynamic range ~ **0.002 – 0.04 IU/ml** in the tested dilution (**ie 0 to 1 IU/ml in plasma using the 1:25 dilution**); flexible working dilution for different assay ranges
- Detection threshold: ~ **0.002 IU/ml** in the tested dilution
- Highly **specific, sensitive, reproducible** (Intra assay CV 2-8 % ; Inter assay CV <9 %)
- **Highly stable** (2 weeks at 2-8 C , 4 days at RT(18-25 C), or frozen).
- **Safe, optimized, standardized:** highly purified human factors and especially **α thrombin**, checked for viral safety
- **No significant interference** of hirudin < 1 µg/ml (for LMWH measurement) and up to < 2µg/ml (for UFH measurement); or of Arixtra® < 2 µg/ml added to plasma.

Summary of Comparison with USP guidelines (proposed IRA for Heparin anti-IIa activity assay)

	~ Kit A221025	~ USP
R1: Human Antithrombin	~0.25 IU/ml in R4 buffer	0.125 IU/ml in buffer (but used at a two fold higher volume)
R2: Human thrombin	α form ~ 25NIH/ml (about 30 nkats/ml) in R4 buffer	5 IU/ml (about 12.5 nkats/ml) in buffer (depending on the thrombin form used)
R3: Thrombin substrate	CS 01-38 (D-Phe-Pip-Arg-pNA) ~1.25 mM.	1.25 mM Thrombin specific substrate
R4: Buffer	Tris 0.05M, NaCl 0.175M, EDTA 0.0075M, BSA 0.2%, pH 8.40	Tris 0.05M, NaCl 0.175M, EDTA 0.0075M, PEG 0-1% and/or BSA 0.2%, pH 8.40
Dynamic range	~0.002 to 0.04 IU/ml	0.005 to 0.03 USP U/ml
Protocol	Microplate: 40µl* specimen 40µl* R1 2 min at 37°C 40µl* R2 2 min 37°C 40µl* R3 1 min 37°C 80µl** acid or kinetics reading (or variant 2: 50µl* and 100µl** if preferred)	Microplate: 50µl specimen 100µl R1 ≥1 min at 37°C 25µl R2 ≥1 min 37°C 50µl R3 ≥1 min 37°C 50µl acid or kinetics reading
Results	Lin-log curve Calculate IU/ml. (Deduce IU/mg).	Lin-log curve Slope ratio or Parallel line assay; USP U/mg

Note: For Biophen kit, the volumes have been harmonized in order to render the assay easier to practice and more reproducible, especially when automated, but concentrations in the final reactive mixture comply with USP recommendations.

Incubation times (especially after R2 addition) are critical and must be strictly adhered to, for optimal performance of the assay.