ANIARA
Manufactured By: HYPHEN BioMed

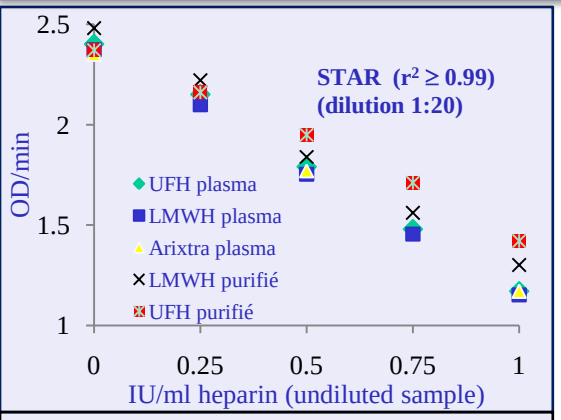
BIOPHEN Heparin anti-Xa (2 stages) technical file (#A221010)

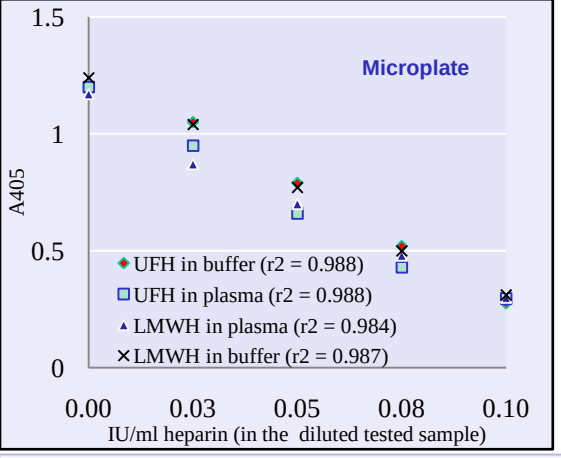
Intended use and applications

☒**RUO:** Determination of heparin anti-Xa 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.] + [FXa (excess)] ➔ [FXa-AT-Hep.] + [residual FXa]
[FXa (residual)] + Substrate ➔ Peptide + pNA

Calibration curve (STAR and microplate)





Related products

1. Biophen Heparin (#A221003/A221006)
2. Biophen Heparin anti-IIa (2 stages) (#A221025)
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 (** ~100 (STAR) to 250 (microplate) tests / kit).
- **Associated plasma calibrators and controls** validated against the International Standard for UFH and LMWH (NIBSC).
- Dynamic range ~ **0.005 – 0.1 IU/ml** in the tested dilution **(ie 0 to 1 IU/ml in plasmausing the 1:10 dilution);** flexible working dilution for different assay ranges
- Detection threshold: **~ 0.005 IU/ml** in the tested dilution
- Highly **specific, sensitive, reproducible (**Intra or Inter assay CV 3-6 % or SD 0.015-0.04)
- **Highly stable (** 2 weeks at 2-8 C , 7 days at RT(18-25 C), or frozen).
- **Safe, optimized, standardized**: highly purified human or bovine factors, checked for viral safety.
- **No significant interference of** hirudin < 2 µg/ml added to plasma.

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

	~ Kit A221010	~ USP
R1: Human Antithrombin	~ 1 IU/ml in R4 buffer	1 IU/ml in buffer
R2: Bovine FXa	~ 8 µg/ml(about 18 nkats/ml) in R4 buffer	~3 nKat/ml in buffer indicative, to be adjusted depending on manufacturer and OD
R3: FXa substrate	CS11-65 (Z-D-Arg-Gly-Arg-PNA) at 0.8mg/ml (~1.2 mM.)	1 mM. FXa specific substrate
R4: Buffer	Tris 0.05M, NaCl 0.175M, EDTA 0.0075M, PEG 0.1%, pH8.40	Tris 0.05M, NaCl 0.175M, EDTA 0.0075M, PEG 0.1% , pH8.40
Dynamic range	~0.005 to 0.100 IU/ml (or 0.02 to 0.5 IU/ml, then diluted in the test)	0.03 to 0.375 USP U/ml (ie 0.006 to 0.075 USP U/ml after dilution in the test)
Protocol (microplate)	**Microplate** 40µl* specimen (***) 40µl* R1 2 min at 37°C 40µl* R2 2 min 37°C 40µl* R3 2 min 37°C 80µl** citric acid 2% (or kinetics) (or **variant 2**: 50µl* and 100µl** if preferred) **Test tube** 200µl specimen 200µl R1 2 min at 37°C 200µl R2 2 min at 37°C 200µl R3 2 min at 37°C 400µl citric acid	**Test tube** 120µl R4 30µl specimen 150µl R1 2min at 37°C 300µl R2 2 min 37°C 300µl R3 2 min 37°C 150µl acetic acid 20% (or kinetics)
Results	Lin-lin curve Calculate IU/ml. (Deduce IU/mg).	Log(A405)- lin (conc) curve Regression best fit and slope calculation; anti-Xa/anti-IIa ratio, Calculate 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. (***alternatively, the specimen can be used more concentrated (range 0.02 to 0.5 IU/ml) by pipetting a five fold lower volume (as per USP protocol) and completing with R4 buffer (ie 10µl specimen and 40µl buffer for variant 2).
Incubation times (especially after R2 addition) are critical and must be strictly adhered to, for optimal performance of the assay.

ANIARA
7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com
www.aniara.com

Form AH111
07-2010
D.750.30/BI/1010 /2 – (Date: Feb 2010)