



## Evaluation Protocol : “Factor XIIa chromogenic assay”

**I/ Aim:**

Proposed protocol for “FXIIa chromogenic assay”, using HBM reagents.

**II/ Reagents and Protocol:**• **Reagents :**

Reagent name	Product reference	Reconstitute with:
Human FXIIa (100ng) (lyophilized)	EZ012A/K	1 ml distilled water
Prekallikrein pool (lyophilized)	PP501B	2ml distilled water
Kallicrein Chromogenic substrate BIOPHEN CS-31(02)	# 229031	<b>Important:</b> 6.7 ml distilled water + 6.7 ml “Tris 0.05M, NaCl 0.15M, pH8.0 specific buffer
« Dilution Buffer »: Tris 0.05M, NaCl 0.15M, BSA 1%, pH 8.0-		

• **Microplate Protocol :**

20µl (OR 50µl\*\*) calibration points (50, 25, 10, 5, 0 ng/ml in the « dilution buffer »)  
or assayed sample

100 µl Prekallikrein pool (HBM ref PP501B)

Incubate for 10 minutes at 37°C

100 µl kallicrein substrate (HBM ref 229031, rec with 6.7ml dist water + 6.7ml « specific buffer »)  
**pre-heated at 37°C**

Read DeltaA405nm between T1=0 min and T2=20 min. (Note\*\*: OR if 50µl specimen is used, alternatively, T2 can be adjusted at 10min, according to the expected OD level).

Express the calibration curve (lin-lin scale) as Delta A405=f (concentration); check that point 0 is on the calibration curve, and that a good linearity is obtained (expected  $r^2 \geq 0.98$ ).

**III/ Expected Results as an indication:**

Sample 20µl / T2=20 min:

Human FXIIa (ng/ml)	50	25	12.5	6.25	0
ΔA405(20min-0min)	1.06	0.57	0.30	0.17	0.02

Sample 50µl / T2=10 min:

Human FXIIa (ng/ml)	50	25	12.5	6.25	0
ΔA405(10min-0min)	1.23	0.63	0.34	0.17	0.01

**Important Comment:** This protocol has been validated in purified milieu. Potential cross reactivities in plasma haven’t been studied. The assay conditions must be chosen in order to make the measurement specific for the targeted enzyme. Provisory protocol: the dynamic range and/or revelation time are susceptible to be adjusted.