



BIOPHEN Plasminogen Technical File

(#A221502)

**Chromogenic assay for the quantitative
determination of Plasminogen
in human citrated plasma.**

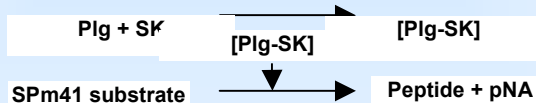
Assay range: 0 – 150 (to 200%) Plasminogen

Apr 2006

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Assay principle

- BIOPHEN Plasminogen is an in vitro chromogenic assay for the quantitative determination of Plasminogen activity in human plasma, using a manual or an automated method.
- Plasminogen (Plg) is the plasma precursor for the fibrinolytic enzyme plasmin, which is generated following plasminogen activation by specific biological activators such as uPA and tPA (in presence of fibrin or fibrin monomers), or pharmacological activators such as streptokinase.
- Plasminogen is measured following its specific activation by addition of streptokinase and plasminogen-free fibrinogen in excess. The complex formed between plasminogen and streptokinase possesses a "plasmin-like" activity, which then specifically cleaves the plasmin-specific substrate SPm41, releasing para-nitroaniline (pNA), which colour is measured at 405nm. There is a direct relationship between colour development and Plasminogen activity in the tested plasma.



Intended use: For in vitro research use only

- Assay of Plasminogen in human plasma for the diagnosis of hereditary or acquired Plasminogen deficiencies.
- An abnormal Plasminogen activity is associated with fibrinolytic dysfunctions.

Kit presentation: 2 x 50 tests (microplate)

R1: Reagent 1: Streptokinase:

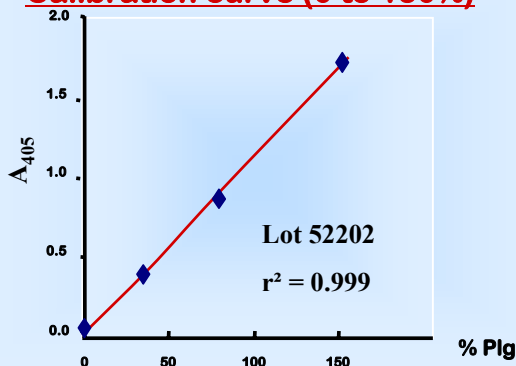
Activation reagent containing streptokinase and plasminogen-free fibrinogen, lyophilized and stabilized (2 vials).

R2: Reagent 2: SPm41 chromogenic substrate, lyophilised (2 vials).

Procedure

- Specimen: citrated human plasma.
- Plasma Dilution: 1:30 (in physiological saline).
- Calibration: plasminogen calibrator, plasma pool or standard.
- End-point method or kinetics protocols.

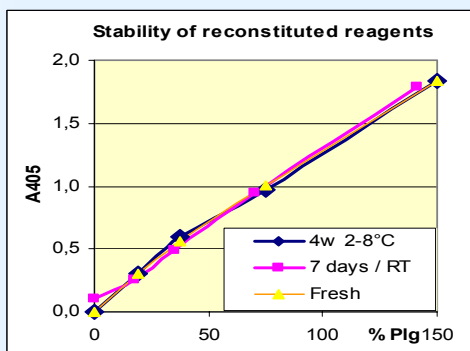
Calibration curve (0 to 150%)



Assay Characteristics

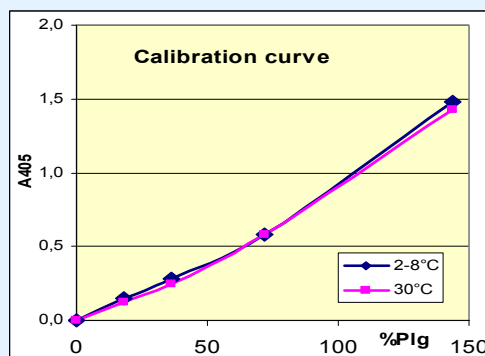
- Total assay time : **10 minutes** or below
- Assay range : **0 to 150 %**
- Reproducibility: $\leq 2 \%$
($N \geq 10$ R1 or R2, tested by manual method on point 100% Plg)
- **Detection limit** (blank+3SD, $N \geq 10$): **< 10 %** (specification $\leq 10\%$)
- **Specificity**: Plasminogen poor plasma **< 6 %** (specification $\leq 10\%$)
- Can be used with: manual, automated, and microplate methods.

Stability of reconstituted reagents



Excellent preservation of performances of reconstituted reagents stored at 2-8°C for 1 month or at RT for 7 days, compared with freshly reconstituted vials.
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Overheating study



Excellent preservation of performances of lyophilised reagents stored for 3 weeks at 30°C or 2-8°C, as compared to freshly restored reagents. Kits can be shipped at RT for a short period without any damage.

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Recovery and accuracy

Goal: To validate the accurate recovery of plasminogen measurements using the BIOPHEN Plasminogen assay, by spiking various concentrations of a normal plasma pool into a plasminogen poor plasma.

Material:

- Biophen Plasminogen: lot 52202.
- Human Plasma Pool (Precision Biologics): Lot A1012 (indicative value: 100% plasminogen), used to establish the calibration curve.
- Plasminogen “poor” plasma (normal plasma, after lysine-sepharose affinity column purification).

Preparation: preparation of plasminogen poor plasma, by addition of various volumes of the normal plasma pool, to obtain expected concentrations ranging from 0 to 100% Plasminogen. Each point is diluted 1:30 in physiological saline for the assay.

Protocol: as per the device insert (manual method)

Results:

Calibration curve		
% Plg	Dil.	A405
150	1:20	1.76
75	1:40	0.80
37.5	1:80	0.35
18.75	1:160	0.15
0	0	0.00

	Expected Plg (%)	Measured Plg (%)
Addition of the plasma pool into plasminogen poor plasma	100% (95%)	95.1%
	90% (86%)	86.8%
	70% (68%)	65.4%
	50% (49.5 %)	50.0%
	30% (31%)	28.4%
	20% (22%)	21.2%
	10% (13%)	13.9%
	0% (4%)	4.0%

Conclusion: Using BIOPHEN Plasminogen, recovery of plasminogen concentrations is satisfactory; a plasminogen poor plasma is measured <10%.

Interferences

Goal: To check that there is no significant interference of various substances in the plasma, at variable concentrations, on the plasminogen measurement using the BIOPHEN Plasminogen assay.

Material:

- Biophen Plasminogen: lot 52202
- Normal human plasma pool (Precision Biologics A1012) considered at 100% Plg, used to establish the calibration curve.
- Various substances: heparin, bilirubin, haemoglobin.

Preparation of tested samples:

individual normal plasmas (different between substances) with addition of the tested substance at different concentrations.

Protocol: as per the STA adaptation.

Results:

Measured Ig	Heparin (IU/ml)				Haemoglobin (mg/ml)						Bilirubin (mg/ml)				
	0	1.0	1.5	2.0	0	1	2	3	5	10	0	2	4	6	
	119	120	119	121	92	90	85	87	88	92	89	90	89	86	
	71	69	69	69	90	94	94	92	91	93	88	87	89	91	93

Conclusion: No significant interference of heparin up to 2 IU/ml in plasma, of haemoglobin up to 2mg/ml, of bilirubin up to 0.2mg/ml.

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Intra and inter assay performances

Results obtained by manual method, using Biophen Plasminogen kit lot 52202:

% Plg	Intra assay			Inter assay	
	Normal pool	Normal control	Abnormal control	Normal control	Abnormal control
1	101.1	97.4	53.8	116.8	50.4
2	101.9	96.0	55.7	100.1	53.9
3	102.7	96.8	53.9	99.3	52.3
4	102.3	95.4	55.2	96.5	54.1
5	99.5	97.4	54.4	94.8	50.5
6	101.5	96.8	55.3	94.0	51.1
7	102.9	94.5	54.8	101.1	57.9
8	104.1	96.4	52.6	101.9	57.0
9	/	97.8	52.6	/	/
10	/	96.2	52.9	/	/
N	8	10	10	8	8
Mean (%)	102.0	96.5	54.1	100.6	53.4
SD	1.29	0.95	1.09	6.7	2.7
CV (%)	1.26	0.99	2.01	6.70	5.00

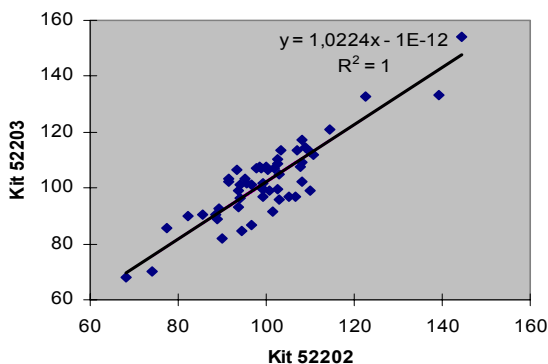
Conclusion: Intra and inter assay reproducibility results are satisfying.

Inter lots performances comparison (manual method)

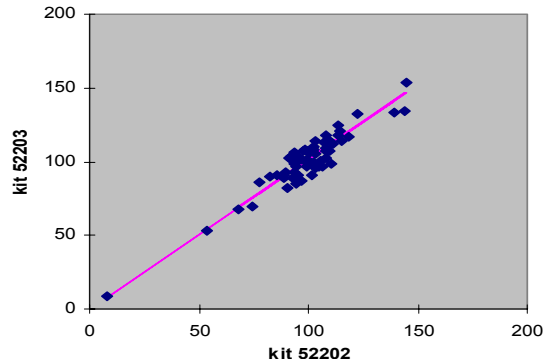
Water bath	Kit 52202	Kit 52203
N (normals)	54	54
Mean (normals)	100.0	102.3
Median (normals)	99.3	101.8
SD (normals)	12.7	14.1
Min	68.3	67.9
Max	144.6	153.9

Water bath	Kit 52202	Kit 52203
N (total)	67	67
Mean (total)	99.4	101.2
Median (total)	99.4	101.6
SD (total)	18.3	19.0
Min	7.6	9.0
Max	144.6	153.9

Biophen PLG: interlots performances analysis by linear regression on normal samples (manual method) (N=54 ; r = 0.998 ; r2 = 0.977; Y = 1.022X)



BIOPHEN PLG: interlots performances comparison by linear regression (manual method) (N=67 ; r = 0.998 ; r2 = 0.981; Y = 1.017X)



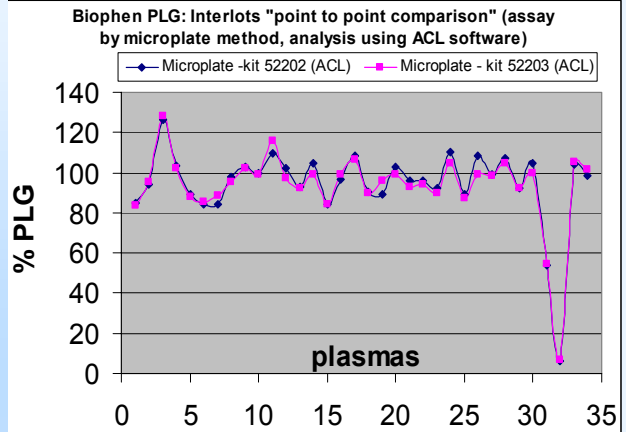
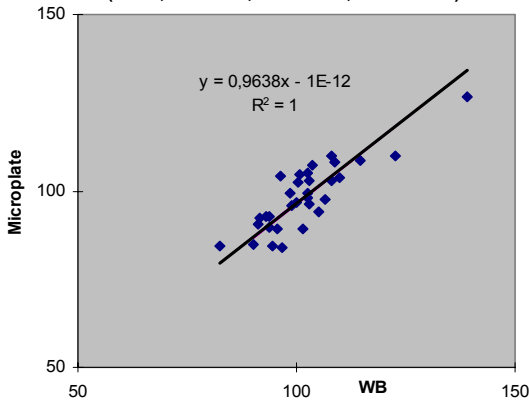
Conclusion: there is a good reproducibility from lot to lot. Inter-lots performances are validated by manual method.

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Microplate method: validation vs manual method & inter-lots performances .

Normal samples	Water bath		Microplate	
	52202	52203	52202	52203
N	32	32	32	32
Mean	101.9	103.7	98.4	97.4
Median	101.0	102.2	98.0	97.9
SD	10.2	11.7	9.3	9.0
Min	82.3	81.8	84.0	83.6
Max	139.1	133.3	126.6	128.1

Analysis by linear regression of Biophen Plg performances (kit 52202) on 32 normal samples: microplate vs water bath method: (N=32; r = 0.998; r2 = 0.964; Y = 0.964 X)



Conclusion:

Excellent correlation between the measured Plg, in %, obtained with the manual or the microplate method. Excellent inter-lots performances with the microplate method. The microplate method is validated.

STA adaptation

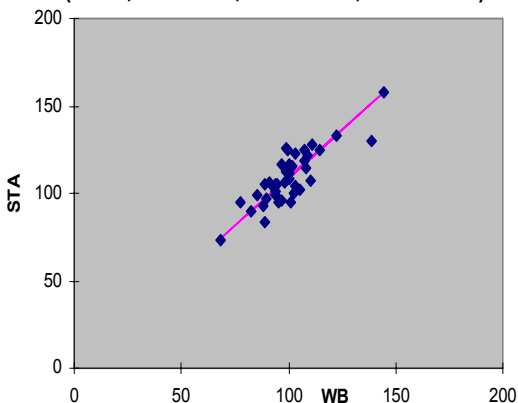
Protocol:

(Refer to D.750.06 (or 07)/BI/1502 for STA):

Calibration defined from 200% (1:10 dilution) to 0%, with an acquisition time of 15-90 sec.

Samples assayed at the 1:20 dilution (by definition, corresponding to "100% plasminogen activity").

Analysis by linear regression : Biophen PLG STA vs Water bath (kit 52202) on 41 normal samples (N=41; r = 0.997 ; r2 = 0.969 ; Y = 1.088X)



	Water bath		STA
	Kit 52202	Kit 52203	Kit 52202
N (normals)	41	41	41
Mean (normals)	100,2	103,0	109,2
Median (normals)	99,2	101,6	106,0
SD (normals)	13,6	14,8	15,2
Min	68,3	67,9	73
Max	144,6	153,9	158

Conclusion:

Excellent correlation between the measured Plg, in %, obtained with the manual method or using STA. The STA adaptation can be proposed.

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Performance comparison of BIOPHEN Plasminogen assay with HemosIL Plasminogen assay for plasminogen activity measurement; normal range.

Goal: Comparison of BIOPHEN Plasminogen performances (manual method & STA) with those of HemosIL Plasminogen assay (ACL) for plasminogen measurement.

Material:

Biophen plasminogen lot 52202 (and lot 52203).

HemosIL Plasminogen lot N0253865

Calibration curve: established with a normal plasma pool (Precision Biologics A1012) (considered at 100% Plg)

Samples:

- Quality controls: Biophen Plasma Calibrator, Normal Control and Abnormal Control.
- Normal plasmas: Precision Biologics (men and women); 2 plasma pools.
- Pathological samples (AVK, thrombosis); Plasminogen poor plasma.

Protocol:

Biophen Plasminogen (manual method):

- Calibration curve: plasma pool (100% Plg) diluted 1:20 ; 1:40 ; 1:80 and 0 in physiological saline. (0 to 150%)
- Working dilution: each sample is tested at the standard 1:30 dilution in physiological saline.

Biophen Plasminogen (STA adaptation):

- Calibration curve: pool (100% Plg) diluted 1:10 ; 1:20 ; 1:40; 1:80 and 0 in physiological saline. (0 to 200%)
- Working dilution: each sample is tested at the "1:20" dilution in physiological saline.

HemosIL Plasminogen (ACL adaptation):

- Calibration curve: plasma pool (100% Plg) diluted 1:20 (considered at 100%).
- Working dilution: each sample is tested at the 1:30 dilution (note: the standard dilution for the test is 1:20, a correction factor x1.5 is then applied).

Results: * Indicative Calibration curves:

HemosIL Plg (ACL)		
% Plg	Dil.	ΔOD
100	1:20	0.623
50	1:40	0.326
25	1:80	0.169
r2	1.00	

BIOPHEN Plg (WB)		
% Plg	Dil.	A405
150	1:20	1.71
75	1:40	0.76
37.5	1:80	0.34
0	0	0.01
r2	> 0.99	

BIOPHEN Plg (STA)		
% Plg	Dil.	OD/mn
200	1:10	1.395
100	1:20	0.677
50	1:40	0.294
25	1:80	0.138
0	0	0.005
r2	0.999	

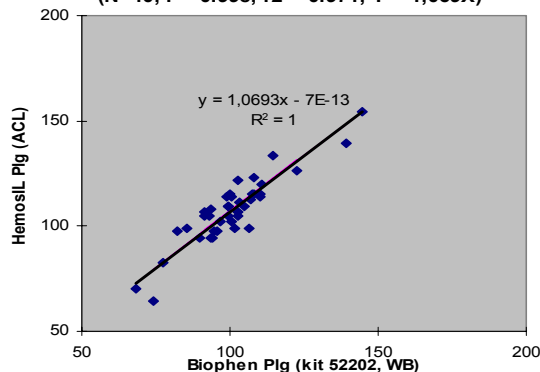
*** Water bath: Plasminogen measurement in normal samples:**

% Plg	Biophen Plg WB/ kit 52202	Biophen Plg WB/ kit 52203	HemosIL Plg (ACL)
N	40	40	40
Mean	100.7	102.2	107.7
Median	100.3	101.8	107.3
SD	14.2	15.7	16.0
Min	68.3	67.9	64.5
Max	144.6	153.9	154.5

The expected normal range of ≈ 70 -130% (mean \pm 2SD) is confirmed.

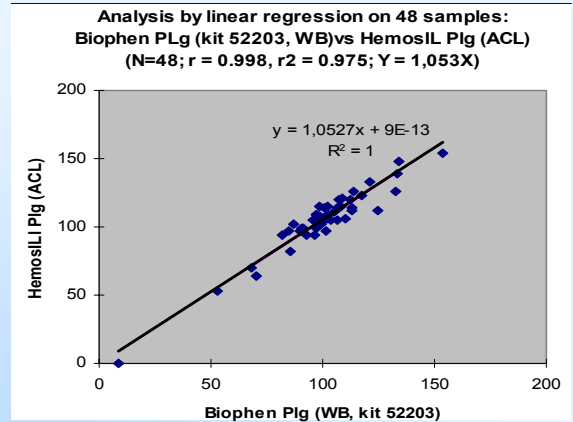
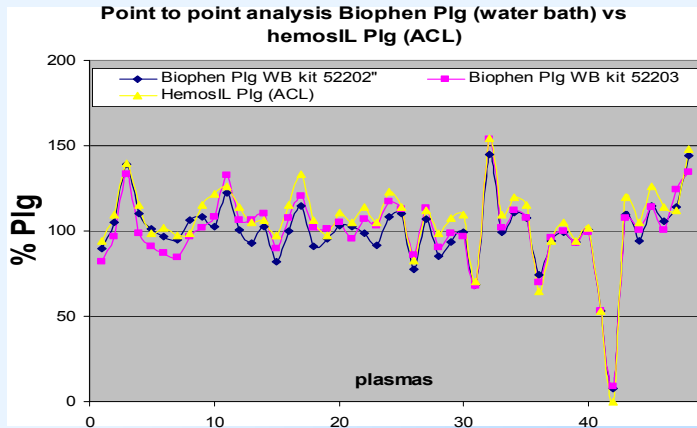
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Performances analysis by linear regression: Biophen Plg (WB, kit 52202) vs HemosIL Plg (ACL):on 40 normal samples (N=40; r = 0.998, r2 = 0.971; Y = 1,069X)



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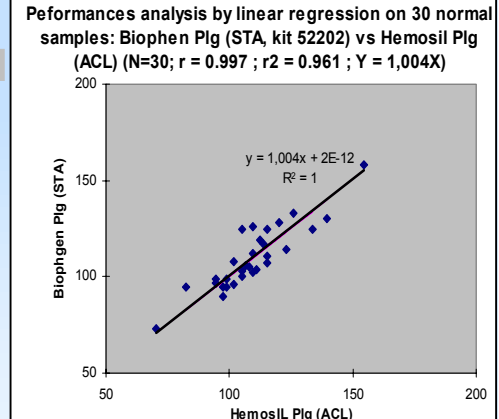
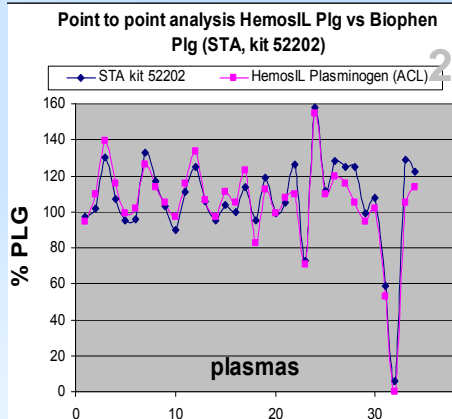
* Water bath : Comparison of Plasminogen measurement in all 48 tested samples:



Conclusion: Good correlation between plasminogen concentrations measured using Biophen Plasminogen kit (water bath) and HemosIL Plasminogen Kit (ACL).

* STA : Comparison of Plasminogen measurement in 30 normal samples:

	HemosIL Plg (ACL)	BIOPHEN Plg (STA)
N	30	30
Mean	109.3	109.9
Median	108.8	106.5
SD	15.9	16.3
Min	70.5	73
Max	154.5	158



Conclusion: There is an excellent correlation between results obtained for 30 normal samples using Biophen Plasminogen kit (STA) and HemosIL Plasminogen kit (ACL).

Clinical applications

By definition, the 100 % Plasminogen concentration corresponds to the concentration in a normal human citrated plasma pool, obtained by pooling plasmas from healthy males or females aged from 18 to 55 years, and out of any medication or disease, diluted 1:30. The Plasminogen concentration in healthy adults is usually in the range 70 to 130% (normal range determined at about 70-130% (mean±2SD) using the BIOPHEN Plasminogen assay, with manual method, on n=54 healthy individuals). Plasminogen concentration is low in neonates. In healthy adults plasminogen level variations are observed with age, smoking habits, pregnancy, hormonal contraceptives, ...

Plasminogen concentration ≤ 50% (in adults) indicates the presence of a deficiency, which must be confirmed with another test and / or by testing another plasma sample from the patient. Plasminogen deficiencies can be: Mostly acquired (they have been observed in hepatic diseases, DIC, sepsis, thrombolytic therapy using plasminogen activators... and in clinical situations associated with hyperfibrinolytic conditions) or hereditary (they can be of type I (hypoplasminogenemia, reduced level of activity and antigen) or of type II (dysplasminogenemia, decreased activity, but normal for antigen levels). They could then be associated with an increased thrombotic risk, still discussed). Ligneous conjunctivitis could also represent a rare but serious complication of plasminogen deficiency. ⇒ **Assay of Plasminogen in human plasma for the diagnosis of hereditary or acquired plasminogen deficiencies. An abnormal Plasminogen activity is an indicator for fibrinolytic dysfunctions.**

References:

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