

Page 1 of 19

Application guide <u>for the Platelet function test by automated aggregation with</u> <u>Epinephrine (AG002K)</u>

This application guide describes the parameters and the procedure for measuring epinephrine-induced platelet aggregation, using the Epinephrine kit (AG002K) for optimal performance on Sysmex CS-5100. This application guide is based on the Sysmex CS-5100 system with the software version 00-13. This document is the only valid source of information for the assay application in the Sysmex CS-5100 system. HYPHEN BioMed has validated the instructions provided for the combination of reagents, instrument, software and customizable features of this system in order to optimize the performance of the product and comply with specifications. The modification of these parameters may affect performance and results. If the application is modified, the user shall be responsible for the validation of the modifications and their impact on all the results. For further information, please refer to the analyzer and reagents instruction manual. A specific configuration is required to perform the test.

1. Test Principle:

When added to platelet-rich plasma (PRP), epinephrine binds to the α_2 -adrinergic receptors on the platelet surface, inducing an initial wave of reversible aggregation. This primary aggregation leads to exposure of fibrinogen receptors and inhibition of adenylate cyclase activity. A second wave of aggregation, clearly distinct from the first, is triggered by ADP release from δ granules and by thromboxane A₂ synthesis. Epinephrine is considered to be a weak agonist as the effects it produces are partial and reversible.

2. Test synopsis:

140 μ L of platelet-poor plasma (PPP) or platelet-rich plasma (PRP) sample 20 μ L of R1 diluted in saline solution Measurement 600 sec at 37°C with mixture OD at 660 nm

Weak/Normal

3. <u>Reagents and material required but not provided:</u>

- Distilled water
- Saline solution (0.9% NaCl)
- 4mL Cup (424-1160-8)
- SB Cuvette (064-1041-9) and SB Kit tool (063-4151-5)
- Laboratory materials and equipment



Page 2 of 19

4. Preparation and stability of reagents:

The stability must be verified and adjusted according to the exact laboratory working conditions.

			Stabi	lity		Position	Name	Reagent
Reagents	Preparation	2-8°C ^[1]	18- 25°C ^[1]	-20°C [2]	On board	on board	of reagent	ID
Epinephrine 40 μmol/L	Reconstitute R1 with exactly 0.625 mL of distilled water. Shake vigorously until complete dissolution. Allow to stabilize 30 min at 18- 25°C shaking the vial occasionally.	7 days	24h	2 months	NA	Reagents Zone ^[3]	Epi Epi2 ^[4] Epi3 ^[4] Epi4 ^[4]	i8 EU EV EW
	Dilute to 1/20 R1 reconstituted in saline solution (1 vol. R1 , 19 vol. saline solution)	NA	NA	NA	10h		Epi4 ^[4] Epi5 ^[4]	EX
Saline solution	Ready to use	NA	NA	NA	NA	Reagents Zone ^[3]	Sal.PPP	FG

^[1]On a closed vial, free of any contamination or evaporation ^[2]Freeze rapidly after stabilization (see freezing/thawing recommendations for lyophilized HYPHEN BioMed reagents). ^[3] It is recommended to transfer to 4 mL cup. ^[4] For multiple concentrations. Do not use a non-validated concentration.

5. <u>Recommendations:</u>

Homogenize well the reagents prior to each use. To obtain a homogeneous reactivity, it is essential to allow the temperature of the reagents stabilize for at least 30 minutes on board the analyzer prior to any use. Do not interchange reagent vials from different batches.

<u>Quality control tests must be performed regularly</u>, and with each change of reagent batch, after any major maintenance work on the analyzer, and when the results do not comply with the expected values for the method. The performance may vary slightly depending on the analyzer used. Each laboratory may establish its own acceptance range.

6. <u>Results:</u>

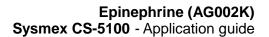
- The results are expressed in % of maximum aggregation.
- It is recommended to make duplicate measurements.

7. Performance and characteristics:

Precision

		Intra-assay	
Samples	n	Mean (% maximum aggregation)	CV (%)
Normal	30	86.0	3.0
Abnormal	30	35.7	7.6

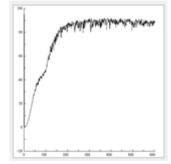
The acceptable variability (imprecision) must be such that the coefficient of variation (CV) of the analytical system on the same normal sample is less than 20% and the abnormal samples less than 30% for the maximum aggregation measurement (%).





Page 3 of 19

Aggregation curve with Epinephrine (For information only)



Range of measurement

Measurement Principle	Analytical measurement range
Aggregation test (optical transmission aggregometry)	0-100% of maximum aggregation

Expected values

The values obtained on healthy subjects vary from one laboratory to another, each laboratory must determine its own reference ranges.

The reference range was established on health adult subjects (n=150) using Sysmex CS-5100, 2000*i*, 2100*i*, 2400, 2500 (Central 90%, 97.5th percentile).

The reference range measured is 68.8 - 99.8 % of maximum aggregation (median: 87.2% of maximum aggregation).

Specificity

The mean value measured out of 150 normal platelet-rich plasmas is 87.0 % of the maximum aggregation.

Interferences

No interference up to ^[1]						
Intralipids ^[2]	ntralipids ^[2] 193 mg/dL					
Hemoglobin ^[3]	100 mg/dL	1 g/L				
Bilirubin (Free)	28 mg/dL	479 µmol/L				
Bilirubin (Conjugated)	28 mg/dL	336 µmol/L				

^[1]Intralipids and Interference Check.A Plus were used in Interference Studies. Clinical samples may exhibit different behavior because Intralipids and Interference Check.A Plus are artificial materials.

Interference Studies have been established using the CS-2000i and 5100 System.

^[2]Platelet Poor Plasma (PPP) samples with high intralipids levels may give an error code [7030.0000.0000 / Ana_PPP_High1]. Accordingly, the associated result may give an error code [7030.0000.0000 / Ana_PPP_High1] and [7050.0000.0000 / Ana_Tube_Position]. The cause of the error code is excess turbidity.

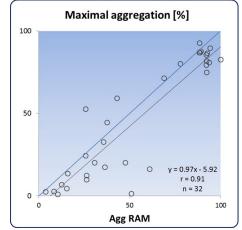
^[3]Refer to ISTH recommendation (Cattaneo, et al, 2013), "hemolyzed samples may affect platelet aggregation and should be discarded."



Page 4 of 19

Comparison of Methods:

The comparison of the methods was established using a CS-2000i system and confirmed for the CS-5100 system.



Epinephrine (Helena) on Helena AggRAM	vs	Epinephrine (HYPHEN BioMed) on Sysmex CS-2000 <i>i</i>	
Number of samples	n	32	
Linear regression	y = 0.97x - 5.92		
Coefficient of correlation	r	0.91	

8. Setup of test:

The special position may contain a maximum of 48 (24x2) cuvettes with magnetic stir bar (SBC) to be inserted with the SBC tool.

Place the PPP and PRP on the sample rack side by side in the order PPP and then PRP. Thus, the PPPs will be in odd numbered positions while the PRP will be in the even numbered positions. Touch the agonist button to start the measurement on the PRP- Once the PPP and PRP analysis has ended, the instruments calculates the percentage of maximum aggregation (% Max aggregation) automatically.

ID Management	Tests	Comments				
8000	PPP	Setup of test for PPP				
8060	Epi	Setup of test for Epinephrine				
8070	Epi2					
8080	Epi3	Setup of test for multiple concentrations				
8090	Epi4					
8100	Epi5					

To program the specific instrument configuration, you should:

1- Select the instrument's English user language via: Menu > Settings > System settings > Language

2- Program the specific configuration according to the settings described below, and check compliance by rereading them.

3- Return to the usual laboratory language via: Menu > Settings > System settings > Language



Page 5 of 19

Assay Group Setup - [PPP]

- ▲ [Main Menu] [Settings] [Assay Group Settings] [PPP]
- ► [Basic]

ic Re-a	nalysis Reflex QC	Test Protocol			
no a					
	Assay Group: PPP		Display Name: PPP	Management ID: 8000	Edit
	📝 Valid		Updated on: 2017/02/07	Assay Group Host ID: 53	
Analyceis	s Condition			Protocol ID: 8000	
	Default Dilution Ratio: 1/1	•			
	Replications: 1 [1]	-			
Assay Pa	arameter				
Valid	Display Name	Assay Param	eter	Updated on	
	PPP	PPP		2017/02/07	
					Сору
					Edit/View
					Delete
					Delete

^[1] It is recommended to perform the tests in duplicate.

► [Re-analysis]

Priority	Assay Parameter	Upper Units	Lower Units	Error Type	Dil. Ratio	Up
						Down
						Add
						Edit
						Delete
	re-analysis Assay Parameter	Upper Units	Lower Units	Error Type	M. Time	Delete
		Upper Units	Lower Units	Error Type	M. Time	Delete
Perform		Upper Units	Lower Units	Error Type	M. Time	

▶ [Reflex]

NOTE: Please adjust the parameters of the Re-analysis conditions, if necessary.

Priority	Comment	Reflex Condition	Dil. Ratio	
				Up Add Down Delet
Reflex Te	st Detailed Settings			
	Priority			Update
	Comment			
	Expression		Edit	
	Dilution Ratio			

NOTE: This tab allows defining the Reflex options. Please adjust the parameters of the Reflex conditions, if necessary.



Edit OC Settings Control: Used in OC Analysis. Stable Time:	Control	Stable Time	Auto QC	Time Interval	Vial QC	Repl.	Dil. Ratio	QC Assay Parameter	
Edit QC Settings									QU
									Down
Control: Used in QC Analysis. Stable Time:	, Edit QC S	Settings							
		Control:		🗌 Used	in QC Analy	sis.		Stable Time:	
Auto QC Analysis Condition	🗖 PPF							Analysis Condition	

NOTE: This tab allows defining the QC options. Please adjust the parameters of the QC conditions, if necessary.

► [Test Protocol]

sic Re-analy			tocol						
Rinse at the Ti Aspiration	ime of Primary	🗖 Pre-R	inse 🗖	Post-Rins	e				
Sample Dispen:	-	🔳 Rinse a	after Buffer Asp	piration					
Dilution Setting Buffer:		▼ Dilutio	n Ratio for Meas	ure ments:	1/1			Edit	
Sample	Samp	le Aspiration Vol	. [uL] Buffer V	olume [uL]	Pre-Rinse List	Post Rinse Lis	st		Add
Sample			140	0	-	-			Edit
									Delet
									Delet
Factor-Deficie	nt Plasma								
Reagent		Vol.	[uL] Pre-Rinse Li	ist	Post Rinse List	t			Add
									Edit
									Lunc
									Delet
Reagent Proto	col								
Reagent Proto Reagent		Probe Type	Pipette at [sec]	Mixing	Pre-Rinse List	Post-Rins	se List	External Rinse	
-	Vol.[uL]	Probe Type Reagent B		Mixing Weak	Pre-Rinse List -	Post-Rins -	se List	External Rinse OFF	Delet
Reagent	Vol.[uL]						se List		Delet Add Edit
Reagent Sal.PPP	Vol[uL] 20						se List		Delet
Reagent Sal.PPP Measurement	Vol[uL] 20 Condition	Reagent B	10	Weak	-	-		OFF	Delet Add Edit
Reagent Sal.PPP Measurement	Vol[uL] 20	Reagent B	10	Weak		-	se List lanagement	OFF	Delet Add Edit
Reagent SalPPP Measurement Measuremen Time(Mair	Vol[uL] 20 Condition	Reagent B Measureme	10 ent Time (Sub): 100 €	Weak	- Seq. Reagent	-	anagement	OFF Stable Time	Delet Add Edit
Reagent SalPPP Measurement Measuremen Time(Mair Measuremen	Vol[uL] 20 Condition nt 100 sec	Respent B Measurement	10 ent Time (Sub): 100 €	Weak	- Seq. Reagent	-	anagement	OFF Stable Time	Delet Add Edit
Reagent SalPPP Measurement Measuremen Time(Mair Measuremen	Vol[uL] 20 Condition nt 100 sec t Time (Main)	Respent B Measurement	10 ent Time (Sub): 100 €	Weak	- Seq. Reagent	-	anagement	OFF Stable Time	Delet Add Edit
Reagent SaIPPP Measurement Measuremen Time(Mair Measuremen ♥ Mixing dur	Vol[uL] 20 Condition nt 100 sec tt Time (Main) M ring measurement	Reagent B Measurement	10 ent Time (Sub): Time (Sub)	Weak Sec	- Seq. Reagent	-	anagement	OFF Stable Time	Delet Add Edit

Assay Parameter Setup - [PPP] ▲ [Main Menu] - [Settings] - [Assay Group Settings] - [PPP] - [Basic] - [PPP]

Assay Parameter Setup						×
Assay Parameter:	PPP	Display Name:	PPP	Management ID:	8000]
	✓ Valid	Updated on:	2017/02/07	Assay Parameter Host ID:	1	



Epinephrine (AG002K) Sysmex CS-5100 - Application guide

Page 7 of 19

► [Calculation Method]

Calculation Metho	Data Check Evaluation Preset
⊚ Raw Data	Unit System: Absorbance 🔹 Units: mOD 🔹 Digits: Integral Part 5 💂 Decimal Part 0 ਵ
⊚ Calib. Curve	Unit System: Absorbance Units: mOD Units: m
	Correction Standard Calibrator Dilution R. Rep. Stable Time Add Edit/View Delete Delete

▶ [Data Check]

Calculation Method Data Check Evaluation Preset	
🔲 Report Limit Check	🗖 Mark Limit Check
Upper Limit: 0 [mOD] Lower Limit: 0 [mOD]	Upper Limit: 0 [mOD] Lower Limit: 0 [mOD]
Replication Difference Limit Check	
Upper Limit: 0 %	
MDA Slope Ratio Check	
Upper Limit: 0.00 Lower Limit: 0.00	



Page 8 of 19

[Evaluation Preset] [Evaluation Parameter]

Calculation Method Data Check	Evaluation Preset	
Evaluation Algorithm Plat	elet analysis 🔻	Detection Principle Aggregation Method 🔹
Target Wave: 660nm 🔹	Smoothing Reference	
Type: Transmitted 🔹	🖉 Median	Preceding Point 10 Following Point 10
Gain: Low 🔹	Moving Average	Preceding Point 4 - Following Point 0
Correction Vuser	Evaluation Parameter E Smoothing	Evaluation Check Parameter Research Parameter
A (Slope) B(Offset)	🗖 Ma×	Preceding Point 2 Following Point 2
1.00 - 0.0000 -	PPP StartTime[sec]:	40.0
V Service	EndTime[sec]:	60.0
A (Slope) B(Offset)	Absorbance At[sec]:	60.0 ¥
1.00 - 0.0000 -	O PRP	
Micro Mode	Start OD StartTime[sec]): 30 🖉 Min OD StartTime[sec]: 3.0 🖉
A (Slope) B(Offset)	EndTime[sec]	: 10.0 × EndTime[sec]: 600.0 ×
1.00 🖉 0.0000 荣	Absorbance At[sec]	
☑ Reagent	● End OD StartTime[sec]]: 5500
A (Slope) B(Offset)	EndTime[sec]	-]: 600.0 ^(A)
	Absorbance At[sec]]: 600.0 🔺

▼ [Evaluation Check Parameter]

Calculation Method Data Check	Evaluation Preset				
Evaluation Algorithm Plat	elet analysis 🔹	Detection Principle	Aggregation Meth	od 🔹	
Target Wave: 660nm 🔹	Smoothing				
Type: Transmitted 🔹	🖉 Median	Preceding Point	10 🜩	Following Point 10 🚍	
Gain: Low 🔹	Moving Average	Preceding Point	4	Following Point 0	
Correction V User	Evaluation Parameter E	valuation Check Par	ameter Research	Parameter	
A (Slope) B(Offset)	High Light Limit:	4000 🜩			
1.00 🗶 0.0000 🖈	Low Light Limit:	50 🖨			
V Service	● PPP ▼ High Level 1:	300 🜩			
A (Slope) B(Offset)					
1.00 💭 0.0000 💭	PRP				
Micro Mode	☑ Low Level 1:	300 🛓			
A (Slope) B(Offset)					
1.00 - 0.0000					
✓ Reagent					
A (Slope) B(Offset)					
1.00 💭 0.0000 荣					



Page 9 of 19

▼ [Research Parameter]

Wave: 660nm Reference Type: Transmitted Image: Construction Preceding Point 10mm Gain: Low Image: Construction Preceding Point 4mm Following Point 0mm Sorrection Evaluation Parameter Evaluation Check Parameter Research Parameter	Evaluation Algorithm Pla	itelet analysis 🔻	Detection Principle	Aggregation Method	•
Type: Transmitted Image: Contraction of the second of	arget	-			
Gain: Image: Transmitted Image: Transmitted <th>Wave: 660nm 🔹</th> <th>Reference</th> <th></th> <th></th> <th></th>	Wave: 660nm 🔹	Reference			
Implified and the second of	Type: Transmitted 🔹	🖉 Median	Preceding Poir	nt <u>10</u> Following Po	int 10 テ
Vlser A (Slope) B(Offset) 1.00 0.0000 0 Evaluation Parameter Evaluation Check Parameter Research Parameter VMax Lag Phase Time Start Time[sec]: 0.0000 0 End Time[sec]: 0.000 0 AUC Start Time[sec]: 0.000 0 AUC Start Time[sec]: 0.000 0 AUC Start Time[sec]: 0.000 0	Gain: Low 🔹	Moving Average	Preceding Poir	nt 4 Following Po	int 0×
A (Slope) B(Offset) 1.00 ÷ 0.0000 ÷ End Time[sec]: 30 ÷ Brop[%]: 250 ÷ AUC Start Time[sec]: 30 ÷ End Time[sec]: 30 ÷ AUC End Time[sec]: 30 ÷ AUC End Time[sec]: 30 ÷ AUC End Time[sec]: 6000 ÷ Abs. difference for 45[mOD]: 200 ÷	Correction V User	Evaluation Parameter E	valuation Check Pa	arameter Research Parameter	
1.00 • 0.0000 • Lag Phase Time 30 • Start Time[sec]: 30 • Result: Find absolute value • End Time[sec]: 600.0 • Start Time[sec]: 2 • Drop[%]: 25.0 • End Time[sec]: 600 • AUC Start Time[sec]: 30 • regwin[sec]: 30 • End Time[sec]: 30 • direct: Decrease • Abs. difference for 45[mOD]: 200 • 20 •	A (Slope) B(Offset)				
End Time[sec]: 600.0 m/m Start Time[sec]: 2 m/m Drop[%]: 25.0 m/m End Time[sec]: 600 m/m AUC regwin[sec]: 30 m/m Start Time[sec]: 30 m/m direct: Decrease End Time[sec]: 6000 m/m Abs. difference for 45[mOD]: 200 m/m					
Drop[%]: 25.0 m/m End Time[sec]: 600 m/m regwin[sec]: 30 m/m 30 m/m AUC Start Time[sec]: 30 m/m direct: Decrease m/m End Time[sec]: 6000 m/m Abs. difference for 45[mOD]: 200 m/m					
AUC StartTime[sec]: 30 EndTime[sec]: 6000 Abs. difference for 45[mOD]: 200 Abs.		EndTime[sec	: 600.0 ਦ	StartTime[sec]:	2 🛓
AUC StartTime[sec]: 30 [±] EndTime[sec]: 600.0 [±] Abs. difference for 45[mOD]: 200 [±]		Drop[%]	25.0 🛬	EndTime[sec]: [600 🌩
StartTime[sec]: 3.0 + direct: Decrease + EndTime[sec]: 600.0 + Abs. difference for 45[mOD]: 200 +				regwin[sec]:	30 🌩
StartTime[sec]: 3.0 + direct: Decrease + EndTime[sec]: 600.0 + Abs. difference for 45[mOD]: 200 +		- AUG			
EndTime[sec]: 6000 × Abs. difference for 45[mOD]: 200 ×]: 3.0 🕀	direct: Deci	rease 🔻
		EndTime[sec			

- Assay Group Setup [Epi] ▲ [Main Menu] [Settings] [Assay Group Settings] [Epi] ▶ [Basic]

Basid	Re-ai	nalysis Reflex QC Test Prot	ocol				
	L	Assay Group: <mark>Epi</mark>		Display Name:	Epi	Management ID: 8060	Edit
		🔽 Valid		Updated on:	2017/03/23	Assay Group Host ID: 55	5 💽
		Condition Default Dilution Ratio: 1/1 Replications: 1 [1]				Protocol ID: 8060	A V
ſ	Assay Pa	rameter					
	Valid	Display Name	Assay Parame	ter		Updated on	
		Epi_min	Epi_min			2017/03/23	
	V	Epi_s	Epi_s			2017/03/23	Сору
		Epi_e	Epi_e			2017/03/23	
							Edit/View
							Delete

^[1] It is recommended to perform the tests in duplicate.



[Re-analysis]

Basic	Re-analysis	Reflex QC	Test Proto	col					
	e								
Pe	erform redilut	ion analysis							
Pric	ority Assa	y Parameter	Upper	Units	Lower	Units	Error Type	Dil. Ratio	Up
									Down
									Add
									Edit
									Delete
🗖 Pe	erform re-ana	Ilysis							
Pric	rity Assa	y Parameter	Upper	Units	Lower	Units	Error Type	M. Time	
									Add
									Edit
									Delete

► [Reflex]

NOTE: Please adjust the parameters of the Re-analysis conditions, if necessary.

Basic Re-a	analysis Reflex QC	Test Protocol		
Priority	Comment	Reflex Condition	Dil. Ratio	
				Up Add
_				
				Down
- Rofloy To	st Detailed Settings			
Treffex Te				Update
	Priority:			
	Comment:			
	Expression:		Ed	lit
	Dilution Ratio: 1	/1 •		

NOTE: This tab allows defining the Reflex options. Please adjust the parameters of the Reflex conditions, if necessary.

▶ [QC]

ontrol	Stable Time	Auto QC	Time Interval	Vial QC	Repl.	Dil. Ratio	QC Assay Parameter		
								Up	Add
								Down	Delete
-Edit QC	Settings Control:		Used	n QC Analy	sis.		Stable Time:		
	ssay Parameter	A	uto QC			Hours	Analysis Condition Dilution Ratio:	·	
E	pi_min pi_s		Auto QC						

NOTE: This tab allows defining the QC options. Please adjust the parameters of the QC conditions, if necessary.



Page 11 of 19

	[Test Protocol]
-	[

sic Re-analys	sis Reflex QC	Test Prote							
linse at the Tin spiration	me of Primary	🗖 Pre-Rin	nse 🔳] Post-Rins	зе				
ample Dispens Dilution Setting	-	🔳 Rinse a	ifter Buffer As	piration					
Buffer: (None	▼ Dilution	n Ratio for Mea	surements:	1/1			Edit	
Sample	Samp	le Aspiration Vol.	[uL] Buffer	Volume [uL]	Pre-Rinse List	Post Ri	nse List		Add
Sample			140	0	-	-			Edit
									Delete
actor-Deficien	nt Plasma								
Reagent		Vol.[uL] Pre-Rinse l	_ist	Post Rinse Li	st			Add
									Edit
									Delete
leagent Protoc									
leagent Protoc Reagent		Probe Type	Pipette at [sec	Mixing	Pre-Rinse List	Pos	st-Rinse List	External Rinse	
-	Vol.[uL]	Probe Type Reagent B		Mixing Weak	Pre-Rinse List -	Po:	st-Rinse List	External Rinse OFF	Delet
Reagent	Vol.[uL]						st-Rinse List		Delete
- Reagent Epi	Vol[uL] 20						st-Rinse List		Delete Add Edit
Reagent Epi Measurement (Vol.[uL] 20 Condition	Reagent B	10) Weak	- Seq. Reagent	-	_ot Management	OFF Stable Time	Delete Add Edit
Reasent Epi Measurement (Measuremen Time(Main,	Val[uL] 20 Condition 1t 600 + sec	Reagent B Measuremen	nt Time 600		-	-		OFF	Delete Add Edit
Reasent Epi Measurement (Measuremen Time(Main,	Vol.[uL] 20 Condition	Reagent B Measuremen	nt Time 600) Weak	- Seq. Reagent	-	_ot Management	OFF Stable Time	Delete Add Edit
Reasent Epi Measurement (Measuremen Time(Main, Measurement	Val[uL] 20 Condition 1t 600 + sec	Rescent B Measurement	nt Time 600) Weak	- Seq. Reagent	-	_ot Management	OFF Stable Time	Delete Add Edit
Reasent Epi Measurement (Measuremen Time(Main, Measurement	Vol[uL] 20 Condition 1: 600 - sec : Time (Main) ▲	Rescent B Measurement	nt Time 600) Weak	- Seq. Reagent	-	_ot Management	OFF Stable Time	Delete Add Edit
Reagent Epi Measurement (Measuremen Time(Main, Measurement I Mixing duri	Vol[uL] 20 Condition 1: 600 = sec : Time (Main) N ing measurement	Reagent B Measurement Measurement T	nt Time 600 (Sub): 600) Weak	- Seq. Reagent	-	_ot Management	OFF Stable Time	Delete Add Edit

Assay Parameter Setup - [Epi_min] ▲ [Main Menu] - [Settings] - [Assay Group Settings] - [Epi] - [Basic] - [Epi_min]

Assay Parameter Setup						×
Assay Parameter:	Epi_min	Display Name:	Epi_min	Management ID:	8060	
	🖉 Valid	Updated on:	2017/03/23	Assay Parameter Host ID:	1	

► [Calculation Method]

Calculation Metho	d Data Check Evaluation Preset
● Raw Data	Unit System: Absorbance 🔹 Units: mOD 🔹 Digits: Integral Part 5 👘 Decimal Part 0 👘
© Calib. Curve	Unit System: Absorbance Units: mOD Units: mOD Digits: Integral Part Decimal Part De
	Edit/View Delete



Page 12 of 19

[Data Check]

🗖 Report Limit Check	Mark Limit Check
Upper Limit: 0 [mOD] Lower Limit: 0 [mOD]	Upper Limit: 0 [mOD] Lower Limit: 0 [mOD]
Replication Difference Limit Check	
Upper Limit: 0 %	
MDA Slope Ratio Check	
Upper Limit: 0.00	

[Evaluation Preset] [Evaluation Parameter]

Calculation Method Data Check		
Evaluation Algorithm Plat	elet analysis 🔻	Detection Principle Aggregation Method 🔹
Target	Smoothing	
Wave: 660nm 🔹	Reference	
Type: Transmitted 🔹	🔽 Median	Preceding Point 10 Following Point 10
Gain: Low ▼	Moving Average	Preceding Point 4 Following Point 0
Correction	Evaluation Parameter F	Evaluation Check Parameter Research Parameter
🔽 User	Smoothing	
A (Slope) B(Offset)	🗖 Ma×	Preceding Point 2 - Following Point 2
1.00 🖨 0.0000 🖨		
	StartTime[sec]:	40.0 🙀
🔽 Service	EndTime[sec]:	60.0 👘
A (Slope) B(Offset)	Absorbance At[sec]:	60.0 m
1.00 ਦ 0.0000 ਦ	PRP	
	💿 Start OD	Min OD
Micro Mode	StartTime[sec]	c]: 3.0 🐳 StartTime[sec]: 3.0 荣
A (Slope) B(Offset)	EndTime[sec]	c]: 10.0 → EndTime[sec]: 600.0 →
1.00 🖨 0.0000 🖨	Absorbance At[sec]	c]: 0.0 🛓
	C End OD	
Reagent	StartTime[sec]	c]: 550.0 ×
A (Slope) B(Offset)	EndTime[sec]	c]: 600.0 👷
1.00 - 0.0000 -	Absorbance At[sec]	c]: 600.0 ×



Page 13 of 19

▼ [Evaluation Check Parameter]

Calculation Method Data Check	Evaluation Preset				
Evaluation Algorithm Plat	elet analysis 🔹	Detection Principle	Aggregation N	Nethod 🔹	
Target Wave: 660nm 🔹	Smoothing Reference				
Type: Transmitted 🔹	🖉 Median	Preceding Point	10 🜩	Following Point 10膏]
Gain: Low ▼	Moving Average	Preceding Point	4	Following Point 0]
Correction Vuser	Evaluation Parameter E	valuation Check Par	ameter Resea	arch Parameter	
A (Slope) B(Offset)	High Light Limit:	4000 🖨			
1.00 - 0.0000 -	Low Light Limit:	50 🗬			
V Service	PPP I High Level 1:	300 🔶			
A (Slope) B(Offset)		0000			
1.00 💭 0.0000 💭	PRP				
Micro Mode	Low Level 1:	300 🛓			
A (Slope) B(Offset)					
1.00 👻 0.0000 👻					
🛛 Reagent					
A (Slope) B(Offset)					
1.00 🜩 0.0000 束					

▼ [Research Parameter]

Calculation Method Data Chec	k Evaluation Preset			
Evaluation Algorithm Pla	telet analysis 🔻	etection Principle	Aggregation Method	•
Target	Smoothing			
Wave: 660nm 🔹	Reference			
Type: Transmitted 🔹	🛛 Median	Preceding Po	int 10 🗧 Following Poin	10
Gain: Low 🔹	Moving Average	Preceding Po	int 4 💭 Following Poin	
Correction	Evaluation Parameter E	valuation Check P	arameter Research Parameter	
A (Slope) B(Offset)	● PRP Lag Phase Time StartTime[sec] EndTime[sec] Drop[%]	: 600.0 🜩	VMax Result: <mark>Find absolute value</mark> StartTime[sec]: EndTime[sec]:	• 2 # 600 #
			regwin[sec]:	30 💌
	AUC StartTime[sec]		direct: Decre	
			Abs. difference for 45[mOD]:	
	EndTime[sec]]: 600.0 ≑		200 荣
			Time difference for 45[sec]:	60 🚔

Assay Parameter Setup - [Epi_s] ▲ [Main Menu] - [Settings] - [Assay Group Settings] - [Epi] - [Basic] - [Epi_s]

Assay Parameter Setup						×
Assay Parameter:	Epi_s	Display Name:	Epi_s	Management ID:	8060	
	🗹 Valid	Updated on:	2017/03/23	Assay Parameter Host ID:	2	



Epinephrine (AG002K) Sysmex CS-5100 - Application guide

Page 14 of 19

► [Calculation Method]

Calculation Metho	Data Check Evaluation Preset
⊚ Raw Data	Unit System: Absorbance 🔹 Units: mOD 🔹 Digits: Integral Part 5 🚔 Decimal Part 0 🐳
© Calib. Curve	Unit System: Absorbance Units: mOD Units: mOD Units: mOD Units: mOD Units: Integral Part Decimal Part Decima
	Graph Axis X Axis Min. : 0 Y Axis Min. : 0 Y Axis Max : 0 Dilution Analysis: * Correction Standard Calibrator Dilution R. Rep. Stable Time Add Edit/View Delete

▶ [Data Check]

Calculation Method Data Check Evaluation Prese	t
Report Limit Check	Mark Limit Check
Upper Limit: 0 [mOD] Lower Limit: 0 [mOD]	Upper Limit: 0 [mOD] Lower Limit: 0 [mOD]
Replication Difference Limit Check	
Upper Limit: 0 %	
🗖 MDA Slope Ratio Check	
Upper Limit: 0.00 Lower Limit: 0.00	



Page 15 of 19

[Evaluation Preset] [Evaluation Parameter]

ameterj				
alculation Method Data Chec	k Evaluation Preset			
Evaluation Algorithm Pla	telet analysis 🔻	Detection Principle	Aggregation	Method 🔹
Target Wave: 660nm 🔹	Smoothing			
Type: Transmitted 🔹	🖉 Median	Preceding Point	10	Following Point 10 두
Gain: Low ▼	Moving Average	Preceding Point	4	Following Point 0
Correction 🖉 User	Evaluation Parameter E Smoothing	valuation Check Par	ameter Res	earch Parameter
A (Slope) B(Offset)	🗖 Ma×	Preceding Point	2 📩	Following Point 2
1.00 🖨 0.0000 束	O PPP			
Service	StartTime[sec]: EndTime[sec]:	40.0 ×		
A (Slope) B(Offset)	Absorbance At[sec]:	60.0 ×		
1.00 🖨 0.0000 🖨	PRP			
Micro Mode	● Start OD StartTime[sec]: 3.0 💌	r <mark>⊚ Min OD</mark> Start	Time[sec]: 3.0 🛬
A (Slope) B(Offset)	EndTime[sec	10.0 🚔	End	Time[sec]: 600.0
1.00 💭 0.0000 荣	Absorbance At[sec	. 0.0		
☑ Reagent	© End OD StartTime[sec	: 550.0		
A (Slope) B(Offset)	EndTime[sec			
1.00 🖨 0.0000 🖨	Absorbance At[sec	600.0 🐳		

▼ [Evaluation Check Parameter]

Calculation Method Data Check	Evaluation Preset			
Evaluation Algorithm Plat	elet analysis 🔻	Detection Principle Aggregation	Method 🔹	
Target Wave: 660nm 🔹	Smoothing			
Type: Transmitted 🔹	🗹 Median	Preceding Point 10	Following Point 10	
Gain: Low 💌	Moving Average	Preceding Point 4	Following Point 0	
Correction Vuser	Evaluation Parameter	Evaluation Check Parameter Rese	arch Parameter	
A (Slope) B(Offset)	High Light Limit:	4000 🚔		
	Low Light Limit:	50 🛓		
	PPP High Level 1: PRP	300 /		
Micro Mode	Low Level 1:	300 🛓		
A (Slope) B(Offset)				
☑ Reagent				
A (Slope) B(Offset)				



Page 16 of 19

▼ [Research Parameter]

Calculation Method Data Chec	k Evaluation Preset
Evaluation Algorithm Pla	telet analysis Detection Principle Aggregation Method
Target Wave: <u>660nm</u> • Type: <u>Transmitted</u> • Gain: <u>Low</u> • Correction V User	Smoothing Reference Median Preceding Point 10 + Moving Average Preceding Point 4 + Following Point 0 + Evaluation Parameter Evaluation Check Parameter
A (Slope) B(Offset)	PRP Lag Phase Time StartTime[sec]: 30 EndTime[sec]: 6000 Drop[%]: 250 AUC
	StartTime[sec]: 30 * EndTime[sec]: 6000 * Abs. difference for 45[mOD]: 200 * Time difference for 45[sec]: 60 *

Assay Parameter Setup - [Epi_e] ▲ [Main Menu] - [Settings] - [Assay Group Settings] - [Epi] - [Basic] - [Epi_e]

Assay Parameter Setup							×
Assay Parameter:	Epi_e	Display Name:	Epi_e	Management ID:	8060		
	🔲 Valid	Updated on:	2017/03/23	Assay Parameter Host ID:	3		

► [Calculation Method]

Calculation Metho	d Data Check Evaluation Preset
● Raw Data	Unit System: Absorbance 🔹 Units: mOD 🔹 Digits: Integral Part 5 🚊 Decimal Part 0 👘
© Calib. Curve	Unit System: Absorbance Vinits: mOD Vigits: Integral Part 5 Decimal Part 0
	Input Value: Calib. Curve Method: Auto Origin Type: Method: Generation
	X Axis: Y Axis: Y
	Extrapolate Range: Min. X 0.80 m - Max X 1.20 m
	Graph Axis X Axis Min. : 0 X Axis Max :0 Y Axis Min. :0 Y Axis Max :0
	Dilution Analysis:
	Correction Standard Calibrator Dilution R. Rep. Stable Time
	Edit/View Delete



Page 17 of 19

▶ [Data Check]

Calculation Method Data Check Evaluation Preset	
Report Limit Check	Mark Limit Check
Upper Limit: 0 [mOD] Lower Limit: 0 [mOD]	Upper Limit: 0 [mOD] Lower Limit: 0 [mOD]
Replication Difference Limit Check	
MDA Slope Ratio Check	
Upper Limit: 0.00 Lower Limit: 0.00	

[Evaluation Preset] [Evaluation Parameter]

Calculation Method Data Chec	k Evaluation Preset	
Evaluation Algorithm Pla	telet analysis	
Target Wave: 660nm ▼	Smoothing Reference	
Type: Transmitted 🔹	Median Preceding Point 10 Following Point	10-
Gain: Low 🔹	Moving Average Preceding Point 4 - Following Point	
Correction Vuser	Evaluation Parameter Evaluation Check Parameter Research Parameter Smoothing	
A (Slope) B(Offset)	Max Preceding Point 2 Following Point	2
1.00 - 0.0000 -	● PPP StartTime[sec]: 400 🗐	
🔽 Service	EndTime[sec]: 60.0 m	
A (Slope) B(Offset)	Absorbance At[sec]: 60.0	
1.00 💭 0.0000 荣	• PRP	
Micro Mode	© Start OD StartTime[sec]: 30♥ StartTime[sec]: 30♥	A v
A (Slope) B(Offset)	EndTime[sec]: 10.0 × EndTime[sec]: 600.0	
1.00 💭 0.0000 🚔	Absorbance At[sec]: 0.0 🖕	
✓ Reagent	● End OD StartTime[sec]: 5500 -	
A (Slope) B(Offset)	EndTime[sec]: 600.0	
1.00 🗬 0.0000 荣	Absorbance At[sec]: 600.0	



Page 18 of 19

▼ [Evaluation Check Parameter]

Calculation Method Data Check	Evaluation Preset				
Evaluation Algorithm Platelet analysis					
Target Wave: 660nm 🔹	Smoothing Reference				
Type: Transmitted 🔹	🗷 Median	Preceding Point	10 🜩	Following Point 1	0
Gain: Low ▼	Moving Average	Preceding Point	4	Following Point	
Correction Vuser	Evaluation Parameter E	valuation Check Para	ameter Resea	rch Parameter	
A (Slope) B(Offset)	High Light Limit:	4000 💭			
1.00 - 0.0000	Low Light Limit:	50			
🔽 Service	O PPP				
A (Slope) B(Offset)	✓ High Level 1:	300 🐳			
1.00 - 0.0000 -	PRP				
Micro Mode	Low Level 1:	300 🔭			
A (Slope) B(Offset)					
Reagent					
A (Slope) B(Offset)					
1.00 🖨 0.0000 🚍					

▼ [Research Parameter]

Evaluation Preset			
elet analysis 🔻	Detection Principle	Aggregation Method 🔹	
Smoothing Reference			
☑ Median	Preceding Po	int 10 Following Point 10	
Moving Average	Preceding Po	int 4 Following Point 0 V	
Evaluation Parameter E	Evaluation Check P	arameter Research Parameter	
EndTime[sec Drop[% AUC StartTime[sec]: 600.0 ¢]: 25.0 ¢	VMax Result: Find absolute value StartTime[sec]: 2 EndTime[sec]: 600 regwin[sec]: 30 direct: Decrease Abs. difference for 45[mOD]: 200 Time difference for 45[sec]: 60	
	elet analysis Smoothing Reference Median Moving Average Evaluation Parameter StartTime[sec Drop[% AUC StartTime[sec	elet analysis Detection Principle Smoothing Reference Wedian Preceding Po Moving Average Preceding Po Evaluation Parameter Evaluation Check P P Lag Phase Time StartTime[sec]: 30 End Time[sec]: 6000 Drop[%]: 250 AUC StartTime[sec]: 30	elet analysis Detection Principle Aggregation Method Smoothing Reference Median Preceding Point 10 + Following Point 10 + Following Point Moving Average Preceding Point 4 + Following Point 0 + Following Point Moving Average Preceding Point 4 + Following Point 0 + Following Point 0 + Following Point Evaluation Parameter Evaluation Check Parameter Research Parameter Evaluation Parameter StartTime[sec]: 30 + Following EndTime[sec]: 6000 + Following StartTime[sec]: 2 + Following AUC StartTime[sec]: 30 + Following Start: Gerease AUC StartTime[sec]: 30 + Following direct: Decrease Abs. difference for 45[mOD]: 200 + Following



Page 19 of 19

Formula setting - [Epi%] [Main Menu] - [Formula setting] - [Epi%]

🖳 Formula Setup				
Formula				
ADP%	Formula Name: Epi%	Display Name: Epi%	Management ID: 18720	Edit
ADP2%	Formula Name. Cpm	Display Name. Epin	Management ID. 10720	Euit
ADP3%	🔽 Valid	Updated on: 2016/01/15	Host ID: 68 崇	
ADP4%	vanu	opdated on. 2010/01/13	HUSE ID. DO	
ADP5%	✓ PlateletFormula		Protocol ID: 18720	
Ara%			110000125.10120	¥
Ara2%	Unit System: Aggregation % 🔹 Unit	t: K 🔹 Digits:	Integral part 4 🌲	Decimal part 1 🗦
Ara3%	Offic Oystem. Aggregation in •		Integrar part 🔽 💽	
Ara4%				
Ara5%	Expression: (([Epi_s]-[Epi_min])/([Epi_s]-[PI	PP]))*100	Edit	
Col% Col2%				
Col3%				
Col4%	Data Check:			
Col5%	🔽 Report Limit Check	Mark Limit Check		
Epi%				
Epi2%				
Epi3%	Upper Limit: 100.0	[%] Upper Limit:	0 [%]	
Epi4%				
Epi5%	Lower Limit: 0.0	16] Lower Limit:	0 [%]	
Ris%				
Ris2%				
Ris3%	QC Settings:			
Ris4%	_			
Ris5%	Control Auto QC Time Interva	al Auto	, QC	
			Auto QC	Hours
				rioure
		Check Rule		
		Settings		
New Up				
Delete Down				0
	Export Add	Load Pri	int Save	Close

Formula

Management ID	Assay Parameter	Formula
18720	Epi%	(([Epi_s]-[Epi_min])/([Epi_s]-[PPP]))*100
18721	Epi2%	(([Epi2_s]-[Epi2_min])/([Epi2_s]-[PPP]))*100
18722	Epi3%	(([Epi3_s]-[Epi3_min])/([Epi3_s]-[PPP]))*100
18723	Epi4%	(([Epi4_s]-[Epi4_min])/([Epi4_s]-[PPP]))*100
18724	Epi5%	(([Epi5_s]-[Epi5_min])/([Epi5_s]-[PPP]))*100