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Australian Government
Department of Health
 Therapeutic Goods Administration

Laboratories Branch

Remember to
add PKA-T AND
PKA-B when ordering!
on 405

Operations Biochemistry - SCS Manual	
Procedure	SCS - 15 - Prekallikrein Activator Assay - Worksheet
Written	s22
Authorised	s22
Date issued	30/09/2016
Revision #	3

SCS - 15 - Prekallikrein Activator Assay- Worksheet

Analyst s22 Date 18 January 2017

Sample Information

All standards, controls and samples should be measured in **REPLICATE**.

Standard:	<u>7th IHS for PKA</u>	Date Calibrated:	<u>14/05/2014</u>	(20/03/14)
LIMS#	<u>1404001560</u>	B#	<u>LE12 MDD1 AC</u>	conc <u>37.2 IU/ml</u> ^{ULF 3} _{16B}
IHC:	<u>7th IHC for PKA</u>	Date Calibrated:	<u>14/05/2014</u>	(20/03/14)
LIMS#	<u>1203001088-R3</u>	B#	<u>LE12 MDD1 AC</u>	conc <u>17.8 IU/ml</u>
Sample 1:	<u>Rhophylac</u>	LIMS#	<u>1608003190</u>	B# <u>4367600001</u> conc <u>150014 (300ug/2ml)</u>
Sample 2:	<u>Intragame P</u>	LIMS#	<u>16100368#10</u>	B# <u>3740501248</u> conc <u>3g/500ul s22 18/01/17</u>
Sample 3:	<u>Privigen</u>	LIMS#	<u>1610003684</u>	B# <u>4346800009</u> conc <u>5g (100g/L) (10%)</u>
Sample 4:		LIMS#		B# conc

Reconstitution of Reagents/Samples

All lyophilised samples and reagents should be brought to room temperature before reconstitution.

Keep all reconstituted samples/reagents at room temp. until use (no longer than 2 hours - Complies ☒ Y / N)

Reagents

Prekallikrein Substrate (**PK Fraction**)(CoaChrom
 Diagnostica, Art#COA0022):

Lot# 549494 Expiry 10/07/17

Working Dilution: 1/5 720ul PK Fraction Stock + 2880ul Buffer B ✓

Substrate S-2302™ (Chromogenix,
 Cat#CX00082034039):

Lot# N1143456 Expiry 10/07/17

Working Dilution: 1/2 use GW 15! 3100ul Subst + 3100ul Buffer B ✓

Buffer B (& "Buffer B Reag") Lot# s22 06 Jan 2017-1 Expiry 6 February 17

CA Clean I (Siemens, Cat# 10445689) Lot# A6060 Expiry 4 February 17
 +
 01-06-2017

Samples/Standard/Control

**** Samples/Standard to be reconstituted as per manufacturer's product information ****

Sample	Concentration (IU/vial)	Volume of ddH ₂ O	Final Concentration (IU/mL)	Appearance (after 15 min)

SCS Pipetting Volume

- Buffer B Rea: (blanks only) $90\mu\text{L} \times 34 \text{ tests} = 3.1 + \text{dv} = 3.6 \text{ mL}$
- PK Fraction: (tests only) $90\mu\text{L} \times 34 = 3.6$
- blanks and tests:
- Chromogenic Substrate (Sub S-2302): $75\mu\text{L} \times 34 \times 2 (\text{blanks}) = 5.1 + \text{dv of line!} = 6.1 \text{ mL}$
- Buffer B: $>0\mu\text{L}$
- Clean I: $>0\mu\text{L}$

Dilutions

- Samples/Standard/Control are to be mixed gently. Do not vortex
- Final volume of dilutions should be no less than **450μL** for 4mL cups and **0.9mL** for 5mL tubes.

For immunoglobulin and albumin samples, dilute to 30g/L (3%) with Buffer B before assay.

Rhophylac	Sample 1:	<u>100</u>	μL of	<u>150</u>	g/L +	<u>400</u>	μL of Buffer B = $30\text{g/L} \times 2 \text{ cups} =$
Integran	Sample 2:	<u>250</u>	μL of	<u>60</u>	g/L +	<u>250</u>	μL of Buffer B = $30\text{g/L} \times 2 \text{ cups}$
Phivigen	Sample 3:	<u>150</u>	μL of	<u>100</u>	g/L +	<u>350</u>	μL of Buffer B = $30\text{g/L} \times 2 \text{ cups}$
	Sample 4:		μL of		g/L +		μL of Buffer B = 30g/L

Select appropriate dilution profile on SCS (eg., N-2-4 for most samples).

See SOP for Standard and Control dilution profiles.

Standard:	SCS dilution profile =	<u>N-2-4-8-16</u>	$\times 2$	<u>= 10</u>
Control:	SCS dilution profile =	<u>N-2-4</u>		<u>= 6</u>
Sample 1:	SCS dilution profile =	<u>N-2-4</u>		<u>6</u>
Sample 2:	SCS dilution profile =	<u>N-2-4</u>		<u>6</u>
Sample 3:	SCS dilution profile =	<u>N-2-4</u>		<u>6</u>
Sample 4:	SCS dilution profile =			<u>total 24</u>

Pipettes Used (LIMS#): 5645 32826 32524

Entered by

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Signed

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QA Checked

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Date

Written By: [Redacted]
Authorised By: [Redacted]
Date issued: 18/09/2015
Revision No: 10

LMS No: 30002
Validated By: [Redacted]
Date Validated: 10/09/2016
Validation Due: 10/09/2017

Sample	Neat Abs	Neat Blank	1 in 2 Abs	1 in 2 Blank	1 in 4 Abs	1 in 4 Blank	1 in 8 Abs	1 in 8 Blank	1 in 16 Abs	1 in 16 Blank
Std 1	0.0216	0.0067	0.0108	0.0033	0.0055	0.0017	0.0027	0.0008	0.0015	0.0003
Std 2	0.0224	0.007	0.0108	0.0034	0.0057	0.0018	0.003	0.0008	0.0016	0.0004
Mean	0.0220	0.0069	0.0109	0.0034	0.0056	0.0018	0.0029	0.0008	0.0016	0.0004
Result (Abs - Blk)	0.0152		0.0075		0.0039		0.0021		0.0012	

IHC 1	0.0134	0.0066	0.0068	0.0032	0.0036	0.0017				
IHC 2	0.0138	0.007	0.0069	0.0034	0.0037	0.0017				
Mean	0.0136	0.0068	0.0069	0.0033	0.0037	0.0017				
Result (Abs - Blk)	0.0068		0.0036		0.0020					

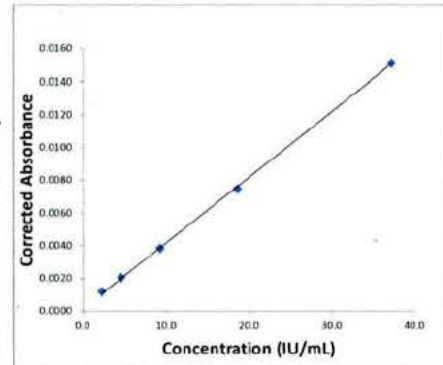
Sample 1-1	0.0014	0.0015	0.0008	0.0007	0.0006	0.0007				
Sample 1-1	0.0014	0.0015	0.0008	0.0007	0.0005	0.0003				
Mean	0.0014	0.0015	0.0008	0.0007	0.0006	0.0005				
Result (Abs - Blk)	-0.0001		0.0001		0.0000					

Sample 2-1	0.005	0.0062	0.0024	0.0029	0.0014	0.0014				
Sample 2-2	0.005	0.0063	0.0026	0.003	0.0015	0.0014				
Mean	0.0050	0.0063	0.0025	0.0030	0.0015	0.0014				
Result (Abs - Blk)	-0.0013		-0.0005		0.0000					

3-1	0.0004	0.0002	0.0004	0.0001	0.0003	0				
3-2	0.0004	0.0002	0.0004	0.0001	0.0003	0				
Mean	0.0004	0.0002	0.0004	0.0001	0.0003	0.0000				
Result (Abs - Blk)	0.0002		0.0003		0.0003					

Sample 4-1										
Sample 4-2										
Mean										
Result (Abs - Blk)										

Conc	Abs	Blank	Abs - Blank	Intercept	0.00017
37.2	0.0220	0.0069	0.0152	Slope	0.00040
18.6	0.0109	0.0034	0.0075	Correlation Coefficient (r2)	0.9997
9.3	0.0056	0.0018	0.0039		
4.7	0.0029	0.0008	0.0021		
2.3	0.0016	0.0004	0.0012		
	0.0136	0.0068	0.0068	Dil'n factor	
	0.0069	0.0033	0.0036	Abs (extr)	
	0.0037	0.0017	0.0020	Blk (extr)	
				Abs-Blk (extr)	
				Manual Cal	
				Average	
	0.0014	0.0015	-0.0001	1	3.06
	0.0008	0.0007	0.0001	2	Out of range
	0.0006	0.0005	0.0000	4	Out of range
				8	
				16	
				Average	
	0.0050	0.0063	-0.0013	1	12.04
	0.0025	0.0030	-0.0005	2	11.81
	0.0015	0.0014	0.0000	4	12.75
				8	
				16	
				Average	
	0.0004	0.0002	0.0002	1	Out of range
	0.0004	0.0001	0.0003	2	Out of range
	0.0003	0.0000	0.0003	4	Out of range
				8	
				16	
				Average	
				1	
				2	
				4	
				8	
				16	
				Average	



Validated
S2
30 JAN 2017

Sample No.	PKA T neat	PKA B neat	PKA T 2	PKA B 2	PKA T 4	PKA B 4	PKA T 8	PKA B 8	PKA T 16	PKA B 16
S1	0.0216	0.0067	0.0109	0.0033	0.0055	0.0017	0.0027	0.0008	0.0015	0.0003
C1	0.0134	0.0066	0.0068	0.0032	0.0036	0.0017				
R1	0.0014	0.0015	0.0008	0.0007	0.0006	0.0007				
I1	0.005	0.0062	0.0024	0.0029	0.0014	0.0014				
P1	0.0004	0.0002	0.0004	0.0001	0.0003	0				
S2	0.0224	0.007	0.0108	0.0034	0.0057	0.0018	0.003	0.0008	0.0016	0.0004
C2	0.0138	0.007	0.0069	0.0034	0.0037	0.0017				
R2	0.0014	0.0015	0.0008	0.0007	0.0005	0.0003				
I2	0.005	0.0063	0.0026	0.003	0.0015	0.0014				
P2	0.0004	0.0002	0.0003	0.0001	0.0003	0				

Checked against screen display

S22

30 JAN 2017

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