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Australian Government

Department of Health

Therapeutic Goods Administration

Office of Laboratories and Scientific Services

## Operations: HPLC Manual

Procedure	HPLC - 01 - General HPLC - WORKSHEET
Written	s22
Authorised	s22
Date issued	DD/MM/YYYY
Revision #	7

## HPLC - 01 - General HPLC - WORKSHEET

TEST DETAILS			
TEST NAME	IgG SE-HPLC		
METHOD REFERENCE	HPLC - 10 - Immunoglobulin SE-HPLC - SOP revision#6		
METHOD MODIFICATIONS (if any)	This worksheet is in draft.		
MODIFICATIONS APPROVED BY:			
NAME OF ANALYST	s22	TEST DATE	19/01/17

## BUFFERS AND SOLUTIONS

SOLUTIONS	BATCH No:
<b>MOBILE PHASE (pH 6.9 ± 0.1) - 2 L</b> 9.746 ± g di-sodium hydrogen orthophosphate dihydrate, Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O 3.482 ± g sodium dihydrogen orthophosphate monohydrate, NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O 23.376 ± g sodium chloride, NaCl In 2000ml ddH <sub>2</sub> O	s22 17 JAN 17 - 1
<b>COLUMN WASH SOLUTION A (pH 3.0 ± 0.1) - 250mL</b> 17.755 ± g sodium sulphate, Na <sub>2</sub> SO <sub>4</sub> Make to 200ml with ddH <sub>2</sub> O, pH to 3.0, make up to 250ml	s22 17 JAN 17 - 2
<b>COLUMN WASH SOLUTION B - 250mL</b> 1.218 ± g di-sodium hydrogen orthophosphate dihydrate, Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O 0.435 ± g sodium dihydrogen orthophosphate monohydrate, NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O Make to 200ml with ddH <sub>2</sub> O, add 50ml of acetonitrile	s22 17 JAN 17 - 3
<b>COLUMN STORAGE SOLUTION - 200mL</b> 20ml methanol Make up to 200ml with ddH <sub>2</sub> O	✓
<b>Sodium Chloride Solution (0.9%)</b> 0.9 g sodium chloride, NaCl Dissolve in 100 mL of distilled water	s22 17 JAN 17 - 4

Record Details	HPLC worksheet - Normal IgG - 170100091, 170100092, 1608003191, 1608003192		
Last Editor	s22	Edit Date	16/01/2017 3:30 PM
Print Date	16/01/2017 4:18 PM		Page 1 of 8

## PIPETTES USED AND EXPIRY DATES

32679 — 10/02/17  
 22018 — ~~27/10/16~~ 20/02/17  
 5648 — ~~05/01/17~~ 16/04/17

## HPLC setup

Column compartment temp:  $21 \pm 10^\circ\text{C}$   
 Sample compartment temp:  $6 \pm 4^\circ\text{C}$   
 Pump flowrate: 0.5 ml/min  
 Sample injection volume: 10  $\mu\text{l}$   
 Detection: UV 280nm (16nm) Ref 360nm  
 (100nm)  
 Run time: 60 min +

Gradient setup: Isocratic / -Gradient

Column ( RP / SE / IEX / other )

TSKgel 3000SW, 600x7.5 mm (Cat#: 05103 ) or TSK gel 3000 SWxl  
 300x7.8mm (Cat#: 08541) columns are suitable.  
 Column# 40, 013, 104, 145, 152

Column storage condition

10% methanol

## Sample set sequence

Injection #	Sample name	Number of injections
<i>Pre-sample sequence</i>		
1	Blank	1
2	Reference standard	3
<i>Sample sequence</i>		
<b>s22</b>		
5	Anti-D – 1608003191	3
6	Anti-D - 1608003192	3
<i>Post sample sequence</i>		
7	Reference standard	3
8	Blank	1
Total number of injection		20

REFERENCE MATERIALS	
NAME AND CODE	BATCH NO:
EDQM Human Immunoglobulin (molecular size)	BRP Batch 1.2
ULF 3-19I	

REFERENCE MATERIAL PREPARATIONS AND CALCULATIONS	
Blank <del>ddH2O</del> MP	
Reference standard – 100 mg/ml (dilute using ddH2O)	
Assigned potency – 700ml/vial	
Reconstitute each vial with 7.0ml of ddH2O	
Storage – 4C for 2 weeks, -80C for 12 months	
	<p>100mg/ml dilute to 40mg/ml w/ 0.9% NaCl</p> <p>— 200µl of 100mg/ml Add <del>400µl</del><sup>300µl</sup> of 0.9% NaCl</p> <p>ie. 20 mg / 0.5ml = 40 mg/ml //</p>

/ATJ  
25 JAN 2017



SYSTEM SUITABILITY CRITERIA AND RESULTS			
PARAMETERS	LIMITS	RESULTS	COMMENTS
Reference standard (n= 6)			
<b>%RSD (System precision)</b> Peak area for the monomer peak of the reference preparation from triplicate injections at the start and at the end, bracketing samples.	s47(1)(b)	0% ✓	rounded to 0 dp. (0.5%) PASS ✓
<b>Relative retention time (RRT)</b> Of the dimer to the monomer peak	s47(1)(b)	0.84 ✓	$= \frac{23.034}{27.440} = 0.84$ ✓
Samples (n= 3)			
<b>Relative retention time (RRT)</b> Of the dimer to the monomer peak <i>relative to the reference std.</i>	s47(1)(b)	1 ✓	PASS ✓
<b>%RSD</b> Peak area for the monomer peak of the <del>reference</del> preparation from triplicate injections		0% ✓	Intragran 170600091 ✓

RT (min)	
	Ref std
dimer	23.0 ✓
monomer	27.4 ✓

ref std s22  
= 1 ✓

= 1 ✓ s22

	Anti-D 1608003191	1608003192
dimer	23.1 (1.00) ✓	23.0 (1.00) ✓
monomer	27.5 (1.00) ✓	27.5 (1.00) ✓

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23/01/2017

Sample criteria acceptable ✓

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s22

## SAMPLE DETAILS

SAMPLE NAME	Human Anti-D Rho Immunoglobulin – Human Immunoglobulin G (IgG) – 625IU (40)		
LIMS No:	1608003191		
BATCH No:	3690850044	EXPIRY:	08/08/2018

## SAMPLE DILUTIONS, CALCULATIONS and DATA

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
17mg/ml (CoA)	✓	✓	40 mg/ml	✓	10 µl 23.5

Dilute sample to 40 mg/ml using 0.9% sodium chloride

## DATA LOCATIONS

Hard copies of Empower reports attached?	YES <input checked="" type="checkbox"/> NO <input type="checkbox"/>	Data location in TRIM	D17-56518
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## TEST RESULTS

PARAMETERS	LIMITS	RESULTS	COMMENTS
Purity		(monomer + dimer) 92 + 5	✓
IgG Monomer and Dimer (Area B)	s47(1)(b)	97% ✓	PASS ✓
Impurity			
Aggregates (Area A)		2% ✓	PASS ✓
Fragments (Area C)		N.D ✓	PASS ✓

SAMPLE RESULTS:

☒ PASS☐ FAIL

Signature of analyst: .....

Date: 23/01/17

Checked by: ..... s22

Date: 23/JAN/2017

Entered in LIMS by: ..... s22

Date: 23/01/17



s22



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Operations Biochemistry – Forms	
Procedure	Forms – Reagent Preparation Record
Written	s22
Authorised	s22
Date issued	20/06/2014
Revision #	3

Reagent Name: <u>Mobile phase (1g6 SEHPLC)</u>	Final Volume: <u>2.0 l</u>
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Prepared by: <b>s22</b>	Date: <u>17/01/17</u>	Expiry Date: <u>17/03/17</u>	Batch #: eg. LB01OCT09-1 <b>s22</b> <u>17JAN17 -1</u>	Storage Temperature: <u>RT</u>
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Component	Manufacturer	Batch #	Required Amount	Measured Amount	Balance / Pipette LIMS #	Final Conc (% , mM, etc)
① <u>Na<sub>2</sub>HPO<sub>4</sub> · 2H<sub>2</sub>O</u> (Sodium phosphate dibasic dihydrate) MW = 277.99 g/mol	<u>Sigma-aldrich</u>	<u>SZB72520</u>	<u>9.746g</u>	<u>9.748g</u>	<u>AA</u> <u>32126</u>	<u>✓</u>
② <u>NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O</u> (Sodium phosphate monobasic monohydrate) MW = 137.99 g/mol	<u>Sigma-aldrich</u>	<u>SLBD729TV</u>	<u>3.482g</u>	<u>3.479g</u>	<u>32126</u>	<u>✓</u>
③ <u>NaCl</u> (Sodium chloride) MW = 58.44 g/mol	<u>Merck</u>	<u>K4575620444</u>	<u>23.376g</u>	<u>23.373g</u>	<u>32126</u>	<u>✓</u>

pH Adjustment	Target pH:	Initial pH:	Adjusted with:	Final pH:	pH Electrode LIMS#: (circle)
	<u>6.9</u>	<u>7.0</u>	<u>HCl / HNO<sub>3</sub> / H<sub>2</sub>SO<sub>4</sub> / H<sub>3</sub>PO<sub>4</sub></u> <u>NaOH / KOH / Other:</u>	<u>6.93</u>	<u>8165 / 32109</u>

Filtration (circle):	None	0.45µm	<u>0.2µm</u>	Other: _____
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Other Comments:	
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**Attach Balance Print Outs Here**

17-Jan-2017 13:22:19  
 → 002: N + 9.748g  
 003: N + 3.479g  
 004: N + 23.373g



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Operations		Biochemistry – Forms	
Procedure	Forms – Reagent Preparation Record		
Written	s22		
Authorised	s22		
Date issued	20/06/2014		
Revision #	3		

Reagent Name: <u>Column wash solution A (IgG SEHPLC)</u>				Final Volume: <u>250 ml</u>		
Prepared by: s22	Date: <u>17/01/17</u>	Expiry Date: <u>17/03/17</u>	Batch #: eg. LB01OCT09-1 s22 <u>17 JAN 17</u>		Storage Temperature: <u>RT</u>	
Component	Manufacturer	Batch #	Required Amount	Measured Amount	Balance / Pipette LIMS #	Final Conc (% , mM, etc)
① <u>Na<sub>2</sub>SO<sub>4</sub></u> <u>(Sodium sulphate)</u> <u>MW = 142.04 g/mol</u>	<u>Merck</u>	<u>K46085437504</u>	<u>17.753g</u>	<u>17.753g</u>	<u>32126</u>	<u>✓</u>
pH Adjustment	Target pH: <u>3.0</u>	Initial pH: <u>7.92</u>	Adjusted with: <u>HC</u> / HNO <sub>3</sub> / H <sub>2</sub> SO <sub>4</sub> / H <sub>3</sub> PO <sub>4</sub> NaOH / KOH / Other:		Final pH: <u>2.97</u>	pH Electrode LIMS#: (circle) <u>8165 / 32109</u>
Filtration (circle):	<u>None</u>	0.45µm	0.2µm	Other: _____		
Other Comments:						
<div style="display: flex; justify-content: space-between; align-items: center;"> <div>Attach E</div> <div>           Here <u>17-Jan-2017 14:39:32</u>  <u>008-N 17.753g</u> </div> </div>						





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Operations Biochemistry – Forms	
Procedure	Forms – Reagent Preparation Record
Written	s22
Authorised	s22
Date issued	20/06/2014
Revision #	3

Reagent Name: <u>Column wash solution B (IgG SEHPLC)</u>	Final Volume: <u>250ml</u>
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Prepared by: <u>s22</u>	Date: <u>17/01/17</u>	Expiry Date: <u>17/03/17</u>	Batch #: eg. LB01OCT09-1 <u>s22 17 JAN 17 - 3</u>	Storage Temperature: <u>RT</u>
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Component	Manufacturer	Batch #	Required Amount	Measured Amount	Balance / Pipette LIMS #	Final Conc (% , mM, etc)
① $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (sodium phosphate dibasic dihydrate)	Sigma-aldrich	SZB92520	1.218g	1.212g	32126	✓
	MW=177.99g/mol					
② $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (sodium phosphate monobasic monohydrate)	Sigma-aldrich	SLBD7295V	0.435g	0.438g	32126	✓
	MW=137.99g/mol					
③ Acetonitrile	Merck	1826530614	50ml	50ml	Measuring cylinder	✓

pH Adjustment	Target pH: <u>✓</u>	Initial pH: <u>✓</u>	Adjusted with: HCl / HNO <sub>3</sub> / H <sub>2</sub> SO <sub>4</sub> / H <sub>3</sub> PO <sub>4</sub> NaOH / KOH / Other: <u>✓</u>	Final pH: <u>✓</u>	pH Electrode LIMS#: (circle) 8165 / 32109
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Filtration (circle):	<u>None</u>	0.45µm	0.2µm	Other: _____
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Other Comments:	
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Attach E

Here

17-Jan-2017 13:55:04  
 005: N + 1.212g  
 006: N + 0.438g



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Operations Biochemistry – Forms	
Procedure	Forms – Reagent Preparation Record
Written	s22
Authorised	s22
Date issued	20/06/2014
Revision #	3

Reagent Name: Sodium chloride solution (0.9%w/v)  
(IgG SEHPLC) Final Volume: 100 ml

Prepared by: s22 Date: 17/01/17 Expiry Date: 17/03/17 Batch #: eg. LB01OCT09-1 s22 17 JAN 17 -4 Storage Temperature: RT

Component	Manufacturer	Batch #	Required Amount	Measured Amount	Balance / Pipette LIMS #	Final Conc (% , mM, etc)
<u>NaCl</u> <u>(sodium chloride)</u>	<u>Merck</u>	<u>K457562044</u>	<u>0.9g</u>	<u>0.898g</u>	<u>32126</u>	<u>✓</u>

pH Adjustment: Target pH: - Initial pH: - Adjusted with: HCl / HNO<sub>3</sub> / H<sub>2</sub>SO<sub>4</sub> / H<sub>3</sub>PO<sub>4</sub>  
NaOH / KOH / Other: Final pH: - pH Electrode LIMS#: (circle) 8165 / 32109

Filtration (circle): None 0.45µm 0.2µm Other: \_\_\_\_\_

Other Comments: \_\_\_\_\_

Attach Balances Here

→ 17-Jan-2017 14:46:21  
 009: N + 0.898g

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