POLYStandard Operating Procedure

Prepare Sample

- 1. Prepare samples as fresh as possible. There is a limited time window before samples start to degrade (molecular weight decreases). This time window will vary sample to sample.
 - a. Can test this for new samples by letting the sample sit in the heated sample chamber, and running the same sample every couple hours to monitor degradation.
- Prepare samples using Tosoh vial and trichlorobenzene solvent. Aim for a 1 mg/mL concentration, anywhere from 5–10 mL total volume. If the solvent level is too low, an error may pop up when running the sample: "A sample cup was not set to the designated cup number."

Note: Solvent does not contain a flow marker. If a flow marker is needed, add a small of any solvent that will not boil at elevated temp (e.g., chloronapthalene, chlorophenol).

3. Seal with foil and special cap.



4. Heat until fully dissolved.

Note: Each sample will require different prep conditions and must be fully dissolved before injecting. As a starting point:

Poly(thiophene): ~145 °C (3-4 hours to fully dissolve) Poly(ethylene): ~135–140 °C (2-5 hours to fully dissolve) Poly(propylene): ~160–165 °C (4-5 hours to fully dissolve)

- 5. After samples are totally finished running and instrument has cooled, clean sample vials, and return vial and cap to [designated storage location]
 - a. Cleaning procedure for sample vials: clean with hot TCB, then THF or an alcohol to remove TCB

Operate Instrument (Warmup, Run/Analyze, Shutdown)

- 1. Computer login: mcneil-group
- 2. Open instrument software by click on the orange "flower" icon on desktop, and selecting blue "flower" acquisition application .



8321GPC-WS	×
Exit Application Help	
User <u>N</u> ame	LabUser 💌
Password	
Application	* *
Log on Lo	g off Close

3. Log on under "labuser" (in drop down list), password: polymer

4. Check solvent level and change out solvent/waste bottles if necessary (instructions in next section)

Instrument control > Instrument parameters tab > solvent stocker > solvent volume

Solvent Stocker	
Temperature control	On
Solvent volume (mL)	0
Waste fluid volume (mL)	4310
Waste fluid warning volume (mL)	3000

5. Turn on pumps

a. Click "instrument control" icon on left side toolbar, and select "instrument parameters" tab.

Power		Analysis	ن Shutdown	Annual acquisition	Ready Ready
旧		Item		Value	^
		Degas level	Std.		
		🖃 Purge			
5		Purge control	Normal		
		Purge volume (mL)	5		
\square		Purge speed(mL/min)	15		
		E Pump Oven			
. . .		Temperature control	On		
		Control temperature (deg. C)	40		
	N	Gas sensor value	300		
833.	IN.				
100 S		Sample column			
	И	Sample pump			
		Flow rate (mL/min)	0.10		
		High limit pressure (MPa)	15.0		
		Low limit pressure (MPa)	0.0		
		E Reference pump			
同		Flow rate ratio	1/1		
ĽQ		High limit pressure (MPa)	15.0		
\square		Low limit pressure (MPa)	0.0		
		E Flow control			
		Sam. pump flow control	Stop.		
		Ref. pump flow control	Stop.	12	
BY		Description Sam. and Ref. pump flow control		<u> </u>	start flow.

b. Under "Flow control", double click "Sam." and "Ref. pump control", then "start flow" button will appear.

🖃 Flow control		
Sam. pump flow control	Flow.	
Ref. pump flow control	Flow.	
Sam, and Ref, pump flow control	Flow.	Stop flow.

- c. Click "start flow". Say "yes" to start flow. Should hear the instrument start flowing, see the pressure increase on the instrument display, and see the solvent lines turn green in the flow diagram view of the software.
- 6. Set up warmup condition (4 hours)
 - a. Click "instrument control" icon on the left side of the screen, and select "warmup" tab.

8321 Project	GPC Acquisition [Instrument]HLC-8321GP	C/HT [Project]Tosof	n Test [User]LabUser - Instrument co	ntrol .	- 🗆 X
Power	Warmup Analysis	Shutdown	Manual acquisition		Ready Ready
自	Warmup condition				
	Pump flow rate ratio:		30 [%]	Current flow rate:0.10[mL/min]	
	Temperature control start:				
M,	Solvent stor	ker	Pump oven	Sampler table	
	J⊄ Injection valv	/e	J♥ Column oven	I detector	
围	Temperature rise rate:		50 [deg. C/h]		
Ð	Stand by at warmup condition	n			
A	Warmup execution time:		120 [min]		
<u>E</u>	Time after warmup end to analyz	e start:	120 [min]		
R					
		\frown		Apply Cancel	
	Flow diagram Instrument Paramet	ters Warmup	Shutdown		
Permissio	User Level 1				

b. Input the following parameters (information is likely already pre-set)

Pump flowrate ratio: 30% Temperature rise rate: 50 °C per hour Warm up execution time: 120 min Time after warmup end: 120 min

All six temperature control boxes should be checked.

The.

c. Initiate warmup sequence by clicking the "warmup" button on the top toolbar.



d. Instrument status (upper right of the top toolbar) should switch from gray "ready" to green "warmup". Flow diagram should be lit up/colored to indicate that solvent is flowing through pumps and ovens are warming up. Entire warm up sequence takes 4 hours. Can make samples and setup sample sequence during this time.



- 7. Set up sample sequence
 - a. Place samples in auto sampler
 - i. Hit "SAM. SET" button (on front of instrument) to unload auto sampler. The autosampler door must be closed with the key turned for this command to function properly.



- ii. Open the autosampler door with key (key should already be in the instrument)
- iii. Place sample vials in numbered tube holders (note they are numbered sequentially spiraling inward) and the close/lock door.
- iv. Hit "SAM. SET" button again (on front of instrument) to reposition the autosampler.
- b. Move the "solvent waste" line in the waste bottle (plastic tube is covered in red rubber tubing).
- c. Click "sample queue" button (vial icon) on the left side tool bar.

wer	Analysis Analysis Contraction	Anual acquisition			Ready Ready
	Sample Queue Wizard		• Ø	New	Save Save As
	Run Time[min]: 0.0 Standard Method: Start Time[min] 0.0 End Time[min]	0.0	Unknown Method Sampling interval	(ms] 0	· ·
Ϋ.,	Cup	ample Name	Injection Volume [uL]	Repeat type	Polarity
222	2				
	5 6 7				
2	8 9 10				
	11 12 13				
(P)	14 15 Standard Method Droffie				

- d. Input sample information and run conditions
 - i. Click "New" and type in sample name in pop-up (note, this sample will not be saved)
 - ii. Input run conditions:

Run time = 35 min Start time = 0 min End time = 35 min Sampling interval = 100 ms Standard method- default Unknown method = default

- iii. Type in cup number (where sample was placed) and give the sample a name (this sample name will be saved with the data).
- e. **Double check** that "solvent waste" line in the waste bottle (plastic tube is covered in red rubber tubing).

f. Under "other settings" you can select whether to "auto balance" (aka zero) the RI just before acquisition or just before the injection. The default setting is "just before data acquisition."

8			Ready
ower	Warmup Analysis Shutdown	Manual acquisition	Ready
<u>ا</u>	Uner settings.	Velue	
	item	Value	
	Auto Balance	Autobalance just before data acquisition	•
a II	Datis of suma flavorate at areas	Autobalance just before injection	
	Auto Savo	Autobalance just before data acquisition	
AA	Naming rule of the database	Auto paming	
7N4	Automatic naming condition		
	Data name		
22	Header	RSIT	
2053	Start number	2	
010	Reset number	M Yes/No	
	Auto Report		
	Auto refresh result screen	S Refresh automatically	
\sim	Acquired signal	RI	
	Column name		
R	Column#1	Col.1	
EQ,	Column#2	Col.2	
	Weekly timer		
	Monday		
L.	Start	Execute	
	Time	08:00	
U M	Warmup	Execute	
苦てし	Stop	Execute	
	Time	20:00	
	Shutdown	Execute	
	- Tuoeday		•
	Description Automatically balancing the aignal just before the data acq	que Bon.	
	see Level 4		

g. Click Analysis button (top tool bar)

	Warmup	Analysis	Shutdown	Manual acquisition						Re
ca	libration standa	urds			-	0	New	Sav	e	Save
	Sample Qu	eue Wizard					Delete	Error C	heck	
-	Run Time[min]	1. 25	Standard Method:	Defent Heller		Unknown Method				
	itan inno[inn]	- 35	Standard method.	Default Method			· D	etault Method		
	Start Time[min	0.0	End Time[min]	35	:	Sampling interval	ms]	100 📩		
	Cup		Sa	mple Name		Injection	Repeat	type	Pola	rity
	1 1	calibrante 1 A-500	E-1 E-20			200	1	Linknown		-
	2 2	calibrants 2 A-100	_F=1_F=20 0 F=2 F=40			300	1	Unknown	+	_
	3 3	calibrants 3 A-250	0 F-4 F-80			300	1	Unknown	+	_
	4 4	calibrants 4 A-500	0 F-10 F-128		•	300	1	Unknown	+	
	5									_
	6									
	7									
	в									
	9									_
	0									_
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	D Dondord Math	ad Brofile	um Mathad Drafila							
	stanuaru metr		wit method Profile							
M	ethod Name:	Default Method								
	r Analytical Con	dition >>								
E E	General]									
-	RI	0.1								
ĸ	alculation Type: appaB [eta]:	1.000	AlphaB [Mv]:	1.000						
-	EXT	Column test								
ĸ	appaB [eta]:	1.000	AlphaB [Mv]:	1.000						

h. The status indicator should turn blue with the text "running" and then "acquisition" once the sample is injected. There is about 7 min between when the sample is marked as "running" and the actual injection. The current sample will be highlighted in yellow ~3 min after the sequence starts.

Before injection:

After injection:

r	W	armup	Analysis	- C Shutdown	Anual acquisition						Running Acquisitio
	calibra	tion standard	is				- 0	New	s	ave	Save As
וע		Sample Qu	eue Wizard					Delete	Erro	r Check	
	R	un Time[min]	35	5.0 Standard Method:	Default Method	 - Unknow	wn Method:	Defau	It Method		
J	s	tart Time[min	1 0	.0 End Time[min]	35.0	Samplin	ng interval(m	s] [100 -		_
		Cup		Sa	mple Name	Injection Volume [uL]	Repeat	type	Polarity		
21	1	1 <mark>- </mark> 0	alibrants_4_A-50	000_F-10_F-128		300	1	Unknown	+		
2	2									-	
3	4									-	
٦H	5										
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21	8									-	
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91	14										
	15 Stan	dard Moth	d Profile	nown Method Profile							
	Metho	vi Name:	Default Method	alown metrod i tome							
- 1	mouro	ra Hame.	Denudic method								
	<< An	alytical Cond	tion >>								
	[Gen	eral]									
	Calcu	lation Type:	Column test								
	Карра	aB [eta]:	1.000	AlphaB [Mv]:	1.000						
	EXT	F	Column toot								
	Kappa	aB [eta]:	1.000	AlphaB [Mv]:	1.000						

i. On the monitor screen, the current sample and time remaining is displayed in the upper left.

8321G	PC Acquisition [Instrument]HLC-8321GPC/HT [Project]Tosoh Test [User]LabUser - Monitor	- 🗆 X
Project	Vonitor Method Sample queue Analysis instrument Report Options Help Image: Construction of the construc	Running Acquisition
B	Image: Remains : 32.97[min] Sample Name : calibrants 1 A-500 F-1 F-20 Image: Total : 35.0[min] Database : 2021-05-13(2).chd Record : 1 / 1 Data Name : RSLT0001	
	Count 1 / 1 Timer Passed Time RI Pressure Sam/Ref. Pump Sam/Ref. Sampler Cup No. Injection Volume Column Line OFF 2.03/35.00[min] -0.06[mV] 1.0/1.1[MPa] Flow(1.00[mL/min]) / Flow(Equal) Ready 1 300 1	
Μ,	STOCKER PO TABLE INJ CO Ri 40.0(40)[deg. C] 40.0(40)[deg. C] 135.0(135)[deg. C] 135.0(135)[deg. C] 135.3(135)[deg. C]	
2003-20 2003-20 2003-20	RI signal Pres. sam. Pres. ref. NJ temp. CO temp. RI temp.	
E	[mM] 10.00	
EQ,		
	5.00	
H	RI autobalance and Labeled injection	
		, , , ,
	PO PUMP SAMPLER CO RI CO RI CO RI CO C Popu	e p Menu
Permission:U	Jser Level 1 X: 1498.38 [min] Y: -0.12 [mV]	//

- j. When analysis is finished, return "solvent waste" line back to the front solvent bottle, wiping tubing with kimwipe to prevent contamination.
- 8. If you are done running samples but want to keep the instrument warmed up for future use, reduce the flow rate to 0.1 mL/min.
 - a. Navigate to the "instrument parameters" tab under the "instrument control" screen.

🔷 8321G	PC Ac	quisition [Instru	ument]HLC-8321GPC/HT	[Project]Tosoh Te	st [User]LabUser - Instrument c	ontrol	_		×
Project N	Monito	or Method S	Sample queue Analysis	Instrument Re	port Options Help			Read	v
Power		⊟ Warmup	Analysis	Shutdown	Manual acquisition			Read	y
			-						
			Item			Value			<u>الا</u>
		Waste fluid	d volume (mL)	35388					-
		Waste fluid	d warning volume (mL)	3000					
	Ľ	Degas Unit		0.1					4.11
		Degas leve	el	Std.					
$ \ge $	Ľ	- Purge	1						4.11
A 4		Purge cont	rol	Normal					- 11
I M		Purge volu	me (mL)	150					- 11
		Furge spee	ed(mL/min)	10					
		- Pump Oven		0.					4
1000		Centrel ter	re control	0n					-
6.5		Gas concer	riperature (deg. C)	900					-
	6			300					
E									1
		Elow	rate (ml/min)	0.10					1
		High	limit pressure (MPa)	3.0					4
		low	limit pressure (MPa)	0.0					-
		Refere	nce pump	0.0					
		Flow	rate ratio	1/1					1
		High	limit pressure (MPa)	15.0					-
		Low	limit pressure (MPa)	0.0					1
		E Flow con	trol						
DWD		Description							<u>.</u>
Öľ		Set Sam. pump 1	flow rate of column 1. (0.10	- 2.00 mL/min)					
		Flow diagram	Instrument Parameters	Warmup Sh	utdown				
Permission:L	Jser Le	evel 1							_

- b. Double click in the "Flow rate" box to edit the flow rate value by typing the desired value and hitting enter. Drop the flow rate in 0.1 mL/min increments, pausing for ~ 30 s at each increment.
- 9. If you are ready to shutdown the instrument (weekends, longer periods without use), initiate the shutdown procedure:
 - a. Click the "instrument control" icon in from the column on the left and navigate to the "shutdown" tab.

💠 8321GP	C Acquisition [In	strument]HLC-8321GPC/H	IT [Project]Tosoh Te	st [User]LabUser - Instrument	control		- 🗆	×
Project M	Ionitor Method	Sample queue Analysi	s Instrument Re	port Options Help				
Power	A Warmup	Analysis	Shutdown	X Manual acquisition			 Read Read	y y
旧	Shutdown con	dition						
	Time aft	er analyze end to shutdown	start:	0 [min]				
	Pump fic	ow rate ratio:		30 [%]	Current flow rate:0.10[mL/min]			
Μ,	Tempera	ature control off:						
		Solvent stocker		✓ Pump oven	Sampler table			
		✓ Injection valve		Column oven	Ri detector			
	Tempera	ature fall rate:		50 [deg. C/	h]			
EQ,	Sampler	wash:						
		Wash execution	Vol	ume: 2.0 [mL]				
ĨΫ		d by at shutdown condition						
	Shutdov	vn execution time:		999 [min]				
	Pow	er off after shutdown end			Apply	Cancel		
	Flow diagram	Instrument Parameters	Warmup Sh	utdown				
Permission:U	ser Level 1							_ //

- b. Set the parameters as follows:
 - i. Time after analysis: 1 min
 - ii. Pump flow rate ratio: 30% (from current flow rate, assumes starting from 1.00 mL/min)
 - iii. Temperature control off: make sure all options are checked
 - iv. Temperature fall rate: 50 °C
 - v. Sample wash: 3 mL
 - vi. Shutdown execution time: 120 min
 - vii. Make sure "standby" is NOT checked (this will turn the flowrate to 0 mL/min)
 - 1. When "standby" is checked pumps stay on and
 - column/detector/injection ovens go to 40 C
 - When "standby is not checked pumps and all ovens turn off
- c. Click "shutdown" from the top toolbar.
 - i. When the instrument is shutdown, the flow diagram should be completely grayed out.
- d. Alternate option: set automatic scheduled shutdown in "other settings."

Analyze Data

1. Open instrument software by click on the orange "flower" icon on desktop, and selecting red "flower" analysis application .



2. Login under "lab user", password: polymer

3. Browse > select user folder > select file > "OK"

- 4. select file > peak edit >
- 5. same process: delete all> draw> sleect baseleine > click before and after peaks > hide front and abck end of calibration
- 6. calculation > edited peak (blue check mark)
- 7. o now the molecular weight data should show up on peak
- 8. o save data
- 9. · lots of options on right hand side
- 10.0 calibration curve RI
- 11. o right click on screen > can copy data, etc. (will export whatever is checked on the right hand side)
- 12. o can highlight all injections, right click > overlay graph
- 13.§ can zoom in
- 14. o results exporter can export data to excel
- 15. o report > second option (title..) > print preview

Upkeep and Maintenance

Changing Solvent Bottle

Note: front bottle is fresh solvent and back bottle is waste.

1. Turn pumps off

a. Under "Flow control", double click "Sam. and Ref. pump control", then "stop flow" button will appear.

Sam, and Ref, pump flow control	Flow.	Stop flow.
Ref. pump flow control	Flow.	
Sam. pump flow control	Flow.	
🖃 Flow control		

- 2. Remove cap from current solvent bottle, take tubing off, and then switch out the old bottle for a new one.
- 3. For waste bottle, must disconnect green wire to remove tubing. Transfer waste into a clear department waste bottle, and return empty amber waste bottle to instrument. Reconnect green wire.
- 4. Perform purge (system icon), *make sure pumps are off!

Purge volume: 150 mL Speed: 15 mL/min for TCB

Start purge process: purge control > double click to get start button on right hand side > start purge

🖃 Purge		_
Purge control	Normal	Start purge
Purge volume (mL)	150	
Purge speed(mL/min)	15	

5. Manually "stop purge after 20 minutes" and then turn pumps back on.

🖃 Purge		
Purge control	Purge is in progress.	Stop purge
Purge volume (mL)	150	
Purge speed(mL/min)	15	

Things to watch out for

- Normal pressure ranges at room temp: ????
- Normal pressure ranges at high temp (135 C): ????
- The RI should be stable before running samples:
 - Drift < 2 mV over 60 min
 - Noise < 0.2 mV</p>
- CAN close the software and the instrument will maintain whatever the most recent flow rate/temp settings are.
- Software gets glitchy if it isn't closed and reopened every now and then.

Yearly Maintenance

- Tosoh sells kit to replace pump hardware (piston, seals, filters etc)
- Can purchase service from Tosoh (tech comes out)
- Bare minimum: replace all the seals on the instrument

Long-term storage

- Shut down instrument
- Remove solvent reservoirs
- Flush solvent from system
- Cap and store columns

Creating a Calibration Curve

- 1. Prepare and run calibration samples
 - a. TSKgel standard PS samples are kept in gold box in small refrigerator



b. Can combine the following samples into the same sample vial (3 mg polymer each/ 9 mL of solvent total):

1: A-500, F-1, F-20 2: A-1000, F-2, F-40 3: A-2500, F-4, F-80 4: A-5000, F-10, F-128

c. Run each sample and save file

- 2. Open data in GPC Analysis software
 - a. Select "chromatogram" from the top toolbar and "Browse chromatogram database" find and open the folder that contains calibration run data. Databases (or sets of sequential sample runs) will save by date acquired
 - i. Chromatogram > browse data> 8321 GPC > sample data > ChromatogramDatabase
 - b. Single left clicking on a database then clicking "OK" will open all the sample runs in that database



3. From the top toolbar, select "Method" -> "New." In the resulting pop-up, name the method whatever you would like and enter a reason. Once you click "OK" the method will show up in the list of available methods at the bottom left.

8321GPC Analysis		-	\Box \times
Chromatogram Method View Graph Calculation	Multi Processing Report Options Help		
		<i>₽</i>	
Save Data SaveAll Data Save Method Result	Method Graph Peak Edit Calculation Multi Processing Re	eport Find	
2021-04-27.chd Browse	Result Chromatogram Information Method Information Instrument In	formation	
Data Name Sample Name	Retention Time Area Height Height%	Half bandwidth Theoretical Resolution Asymmetry	
RSLT0003 calibrants_1_A-500_F-1_F-20	[min] [mV*s] [mV] [%]	[s] Plates Factor Factor	
RSL10002 calibrants_3_A-2500_F-4_F-80 RSL10001 calibrants_2_A-1000_E-2_E-40			
K3210001 Calibranta_2_X+1000_1+2_1+40			
	•		•
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	[mV]	Chromate	ogram RI
		RI M Chromato	ogram EXT
	Create New Method	X Peak # Elution Co	urve RI
	Method Name calibration 05-14-2021	Top Molecular Mass Calibratio	in Curve RI
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		Temperat	ture CO
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Method with Chromatogram			
calib-practice			
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	-10.000-		
	I I I I I I I I I I I I I I I I I I I		
	0.000 10.000 [min]	20.000 30.000	
Method Property			
Logon User : LabUser (User Level 1)	Project : Tosoh Test		
8321GPC Analysis		_	
 8321GPC Analysis Chromatogram Method View Graph Calculation 	Multi Processing Report Options Help	-	0 X
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S321GPC Analysis Chromatogram Method View Graph Calculation Swe Data SaveAll Data Save Method Result 2021-04-27 chd Data Name Calibrants 1, A-500_F-1, F-20 RSLT0003 calibrants 1, A-500_F-4, F-80 RSLT0001 calibrants 2, A-1000_F-2, F-40	Multi Processing Report Options Help Method Graph Peak Edit Calculation Multi Processing R Analytical Condition 1 Analytical Condition 2 Peak Edit Condition Calculation Calculation Type KappaB [eta] AphaB [Mv] ElexT Calculation Type KappaB [eta] AphaB [Mv]		
◆ 8321GPC Analysis Chromatogram Method View Graph Calculation Swe Data SaveAll Data Save Method Result 2021-04-27 chd ■ Browse. Data Name Sample Name RSLT0003 calibrants _1.4-500_F-1.JF-20 RSLT0002 calibrants _2.A-1000_F-2_F-40 RSLT0001 calibrants _2.A-1000_F-2_F-40	Multi Processing Report Options Help Method Graph Peak Edit Calculation Multi Processing R Analytical Condition 1 Analytical Condition 2 Peak Edit Condition Cal Rem Analytical Condition General Ref Calculation Type KappaB (eta) AlphaB [Mv] Calculation Type KappaB (eta) AlphaB [Mv] Output Type	Poport Find Value Column test 1.0000 1.0000 Column test 1.0000 Area	
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4. To view the chromatograms, select "Graph" from the upper toolbar

5. Click the sample you wish to analyze from the list at the upper left



6. Click the "peak edit" icon to open the window where you can edit the chromatogram



7. Click "delete all" to remove any auto-generated peaks/baselines.



8. Select "Draw" then left click and drag to select the baseline. A green line will appear once the baseline is set.



9. Single left click to set the peak limits. Numbers will appear above each selected peak.



10. Additional peaks will likely appear to the left and right of the true polymer peaks. To remove these peaks from the list of polymer peaks, select "hide peak." Hovering over different regions will highlight them in black. Single left click on the "peaks" you wish to remove.



11. Click "calculation" from the top toolbar and select "Edited Peak." A results table will appear and the sample name will be marked with a blue check mark. Repeat steps 6-11 for all samples that you wish to include in the calibration curve.



12. Select "Save All Data" and enter in a reason (whatever you like) in the resulting pop-up window. Click "OK."

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Chromatogram Method View Graph Calculation	Multi Processing	Report Optio	ns Help								
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RSLT0002 calibrants_3_A-2500_F-4_F-80	1 RI										
RSLT0001 calibrants_2_A-1000_F-2_F-40	2	8.198	487.725	12.339	55.15	56 30	37.257	965	1 207	1.326	
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 Select "Method." Under the "Analytical Condition 1" tab, double click the value for "RI -> Calculation Type." From the resulting dropdown menu, select "Molecular Mass."

💠 8321GPC Analysis				- 🗆 ×
Chromatogram Method View Graph Calculation	Multi Processing Report Options Help			
Save Data SaveAll Data Save Method Result	Method Graph Peak Edit Calculation	Multi Processing Report	₽ Find	
2021-04-27.chd Browse	Analytical Condition 1 Analytical Condition 2	Peak Edit Condition Calibration Conditio	n)	
Data Name Sample Name			1	
RSLT0003 calibrants_1_A-500_F-1_F-20	Item		Value	^ _
RSLT0002 calibrants_3_A-2500_F-4_F-80	Analytical Condition			
RSLT0001 calibrants_2_A-1000_F-2_F-40	General			
	R			
	Calculation Type	Molecular Mas	iS	-
	KappaB [eta]	Column test Molecular Mas	16	
	AlphaB [Mv]	Copolymer		
	EXT			
	Calculation Type	Column test		
	KappaB [eta]	1.0000		
	AlphaB [MV]	1.0000		
	Output Type	Area		
	Correction by Internal Standard Beak			
	Patantian Time [min]	0.000		
	Panae [min]	0.000		
	Calculation Internal Standard Beak	Reject		
		10,000		
	Correction by Internal Standard Peak	Yes		
	Retention Time [min]	0.000		
Method with Chromatogram	Range [min]	0.000		
✓ calibration_05-14-2021_practice	Calculation Internal Standard Peak	Reject		
calibration_05-14-2021	Correction by Lag Time	Yes		•
calib-practice	Description			
Default Method	The parameter is the calculation type of RI.			
Method Property				
Logon User : LabUser (User Level 1)	Project : Tosoh Test			1.

14. Under the "Calibration Condition" tab, select "Set the Retention" to add the data from your calibrants.

B B E E Save Data Save All Data Save Method Result	Method Grap	Peak Edit Ca	lculation M	alti Processing 👻	Beport	▼ Find		
021-04-27.chd Browse	Analytical Condition	1 Analytical Co	ndition 2 Peak	Edit Condition	alibration (Condition		
Data Name Sample Name		-						the Detection of
RSLT0003 calibrants_1_A-500_F-1_F-20	IRI .	Approximation	Linear: At+B				<u> </u>	set the Retenuor
RSLT0002 calibrants_3_A-2500_F-4_F-80		Correction :	Non					Redraw
RSLT0001 calibrants_2_A-1000_F-2_F-40	Calibration Data							
	Time [min]	Molecular Mass	Correction Value	Approximation	Error	Weight _ [LogM]		Error[%]
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	12					0.000	5.000	10.000
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Method with Chromatogram	Correlation Coerr	icient.	0.000	C :		0.00000000		
calibration 05-14-2021 practice				D :		0.00000000		
calibration 05-14-2021				E:		0.00000000		
calib-practice				6		0.000000000		
Default Method				н:		0.00000000		

15. In the resulting pop-up, click "Registration" to find calibration data.

Data

16. Find the calibrant sample runs that you wish to add to the calibration curve. For each sample, click to select it (on the left), then hit apply. Double check that the sample is added to the registration window before adding subsequent samples.

Registered Data Name		Database	Message	9
RSLT0001	(C:\8321GPC\Data\Tosoh	Test\LabUs	
Registration	Create	e from Memory Data	1	
Dalata	Creat	from Another Data	1	Class
Delete	Create	e from Another Data]	Close
t the Chromatogram				- 0
ndows (C:)	^	Data Name	Sample Name	Acquisition Date
8321GPC		RSLT0001	calibrants_4_A-5	2021/05/12 10:51
Converter				
🔲 Data 🛛 🗖 🔲 🔲	ases	II ▲		
- Tosoh Test				
LabUser		sample ri	ins	
2021-04-26		Jumpien		
2021-04-27				
2021-05-11				
2021-05-12				
2021-05-12/	2)			
2021-05-12	-/			
2021-05-13	2)			
2021-00-13(e)			
Torob Validations	mato			
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Documents				
Documents Driver				
Documents Driver Log				
Documents Driver Log Report				
Documents Driver Log Report Resource				
Documents Driver Log Report Resource SampleData	~			

17. Once you have added all of the calibration samples you desire, click "close." In the registration window, click "Create from Another Data." The word "completed" will auto-populate the right hand column. Click "Close."

Registered Data Name	Database	Message
RSLT0001	C:\8321GPC\Data\Tosoh Test\LabUs	Completed.
RSLT0001	C:\8321GPC\Data\Tosoh Test\LabUs	Completed.
RSLT0002	C:\8321GPC\Data\Tosoh Test\LabUs	Completed.
RSLT0003	C:\8321GPC\Data\Tosoh Test\LabUs	Completed.
	1	
Registration	Create from Memory Data	

18. The retention times from all of your calibrants should appear in the "Time" column under the "Calibration Conditions" tab.

8321GPC Analysis	-	
Chromatogram Method View Graph Calculation	Multi Processing Report Options Help	
Save Data SaveAll Data Save Method	Image: Construction of the second	
2021-04-27.chd Browse	Analytical Condition 1 Analytical Condition 2 Peak Edit Condition Calibration Condition	
Data Name Sample Name		
RSLT0003 calibrants_1_A-500_F-1_F-20	RI Approximation : Linear: At+B	t the Retention
RSLT0002 calibrants_3_A-2500_F-4_F-80	Correction : Non	Redraw
RSLT0001 calibrants_2_A-1000_F-2_F-40	Calibration Data	
	Time [min] Molecular Mass Correction Value Approximation Error Weight 📥 [LogM]	Error[%]
	2 7.978	
	3 8.198	
	4 8.425	
	5 8.688	
		- 0
	9 9 985	
	10 10.323	
	11 10.667	L /
	12 10.755	
		10 000
	Time[min]	10.000
	Approximation Corrections Options	
		11
	Cutoff (L): 0.000	
Method with Chromatogram	Correlation coefficient 0.000 C : 0.00000000	
✓ calibration 05-14-2021 practice	D: 0.00000000	
calibration 05-14-2021	E: 0.00000000	
calib-practice	F: 0.00000000	
Default Method	G: 0.0000000	
	H : 0.00000000	
Method Property		
Logon User : LabUser (User Level 1)	Project : Tosoh Test	1

19. From the top toolbar, click "Options" and select "Molar Mass Registration" to open a database of standard data.

8321GPC Analysis							- 🗆 ×
Chromatogram Method View Graph Calculation	Multi Processing Report Op	tions Help					
		Molecular Mass Reg	istration		• P		
Save Data SaveAll Data Save Method Result	Method Graph Peak	Comment Registrati	on	Report	Find		
2021-04-27.chd		Graph Color					
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Data Name Sample Name		orapiti onc				•	Set the Retention
RSLT0003 calibrants_1_A-500_F-1_F-20		Toolbar		<u> </u>			Dadaau
RSLT0002 calibrants_3_A-2500_F-4_F-80	Correct			-		•	Redraw
RSLT0001 calibrants_2_A-1000_F-2_F-40	Calibration Data						
	Time [min] Molecular	Mass Correction Valu	e Approximation	Error	Weight _ [LogM]		Error[%]
	1 7.688				2		50
	2 7.978						F 1
	3 8.198						
	4 8.425				1		
	5 8.688				1		
	6 9.043				1-		- 0
	7 9.337						
	9 9985				- 1		
	10 10.323						LI
	11 10.667				-		
	12 10.755						
	13 12.378					5 000	10 000
	•				•	Time[min]	10.000
	Approximation Correction	Dotions					
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Method with Chromatogram			D:		0.00000000		
 calibration_05-14-2021_practice calibration_05-14-2021 			E:		0.000000000		
calibration_05-14-2021			F:		0.00000000		
Calib-practice			G :		0.000000000		
Default Method			H :		0.000000000		
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Method Property							
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20. Select the appropriate group of standards from the list on the left. Our lab uses "TSK Polystyrene Standards." In the "valid" column near the center, click each standard that was used to generate the calibration curve. Selected standards will have a red check mark. Click "OK."

51		_				
PStQuick A	<u> </u>	Valid	Grade	Lot No.	Molecular Mass	Mw/Mn
PStQuick B	7		F-288		2890000	1.09
PStQuick C	8		F-128		1090000	1.08
PStQuick D	9		F-80		706000	1.05
PStQuick E	10		F-40		427000	1.02
PStQuick F	11		F-20		190000	1.0
PStQuick Kit-H	12		F-10		96400	1.0
PStQuick Kit-L	13		F-4		37900	1.0
PStQuick Kit-M	14		F-2		18100	1.0
PStQuick MP-H	15		F-1		10200	1.0
PStQuick MP-M	16		A-5000		5970	1.0
PStQuick MP-N	17	5	A-2500		2630	1.0
TSK Standard Polystyrene	18		A-1000		1050	1.1
TSK Standard Polystyrene Oxi	19		A-500		495	1.1
			A 200		452	

21. Double click on the first box in the "Molecular Mass" column and select "Setting Values" from the resulting dropdown menu.



22. The software should auto-populate the rest of the table with known data from the standards that you just selected and should auto-calculate the calibration curve. The error values for a good calibration curve should be less than 10. A red checkmark will now be next to the calibration method name at the lower left.

8321GPC Analysis					- 🗆 X
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Save Data SaveAll Data Save Method Result	Method Graph	Peak Edit Calculation	Aulti Processing Re	eport Find	
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Data Name Sample Name	-				
RSLT0003 calibrants_1_A-500_F-1_F-20	RI	Approximation : Linear: At+B			Set the Retention
RSLT0002 calibrants_3_A-2500_F-4_F-80		Correction : Non			✓ Redraw
RSLT0001 calibrants_2_A-1000_F-2_F-40	Calibration Data				
	Time [min]	Molecular Mass Correction Value	Approximation	Error Veight 🔺 [LogM]	Error[%]
	1 7.688	1090000 1090000.	10 1205799.35	-10.02	- 50
	2 7.978	706000 706000.	0 597432.96	15.38 1 6-	א_ דו
	3 8.198	427000 427000.	0 350687.74	17.87 1	Yo -
	4 8.425	190000 190000	202390.57	-6.52	
	5 8.688	96400 96400.	10/053.20	-11.05	
	7 9.043	18100 18100	10 40010.04	-19.57	/++++++++
	8 9.653	10200 10200	10345.06	-1 42	
	9 9.985	5970 5970.	4629.95	22.45	
	10 10.323	2630 2630.	0 2042.26	22.35 3	
	11 10.667	1050 1050.0	887.84	15.44 1	
	12 10.755	495 495.	0 717.44	-44.94 1	50
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23. Click "Save Method" and "OK." The method is now ready to use!

8321GPC Analysis								– 🗆 🗙
Chromatogram Method View Graph Calculation	Multi Processin	a Report Options	Help					
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Logan liner : Labliner (liner Lavel 1)		Project - Topoh Te	et					

Other Information

Some key differences between the high-temp system and a typical SEC system:

- The instrument is designed to be turned on and off without ruining the columns
 - Less frequent on/off the better (i.e., turn on for M-F, off for weekends rather than on/off every day)
- Instrument needs ~2h warm up/cool down to power on/off
- Might see higher pressure on the instrument readout because the solvent is more viscous at lower temperatures (at operating temp of 135 C, the pressure should be lower)
- Instrument has several ovens to heat the different parts surfaces can be hot while it is turned on!
- Solvent (1,3,5-TCB) can degrade with heat (gels and releases HCI), tubing will start turning red
 Flush the instrument with new solvent every 1 or 2 months
- Should turn on the instrument ~1/wk to check that things are running smoothly

Manual control of systems parts

"Monitor" screen

- When you open the software, should see "monitor" screen by default
- Look for grey "ready" (as opposed to "disconnect")
- Real time RI signal is in blue, pressure is green, reference pressure is dark green
 - Monitor -> monitor settings to see other signals
 - Left click and drag to translate, right click to zoom
- Check the pressure should be 2 mL/min bc running 1 mL/min thru both columns

"Flow diagram" screen

- Shows flowchart of the whole instrument, can control individual parts from here by clicking
 - 6 heating regions (orange)
 - Live solvent flow (green)
- Solvent reservoirs should be kept at 40 C at all times
- The RI detector and the columns should always be kept at the same temperature
- The sample holder can be kept at a lower temp if desired (prevent sample decomposition for long autosampler runs)
- There's a gas sensor on this screen 300 (?? not sure what this means) to detect leaks
 - Tighten the lower pressure sensor limit as you use the instrument
 - Record the pressure at 0.5 mL/min (which is the warm-up flow rate)
 - \circ $\,$ 1 or 2 mPa lower than the above pressure is the lower limit
 - The high pressure limit should be listed on the column spec sheet

General instrument things:

- Two solvent bottles fresh (front), waste (back)
 - Solvent is degassed before entering the system
 - When replacing solvent, perform 150 mL pump purge (to take care of any air exposure)
- Two feeds for solvent into the instrument (column 1 = sample, column 2 = reference)
- Three feeds for solvent out of the system (columns + autosampler/injection waste)
 - Autosampler/injection waste reservoir is 300 mL, keep an eye on it
- Uses dual pump system for pressure stability
- Uses two columns, a sample column and reference column (for the continual flow RI reference cell)
- Two sets of keys are available to lock the instrument doors

Software

The analysis software can be downloaded onto personal computers! Username: LabUser Password: polymer

- 1. Blue icon = live instrument software
- 2. Red icon = offline software (data workup)
- 3. Teal icon = database manager