

Test plate

Tuesday, April 27, 2021 7:58 PM

TEST PLATE	1	2	3	4	5	6	7	8	9	10	11	12
A	Batch Ref (VP)	141171	141201	613	151280	141361	1231	1095	141374	Batch Ref (VP)	141345	1091
B	Batch QC (GW)	1207	141232	587	141335	1116	Batch Ref (VP)	1030	1197	151068	1014	141112
C	687	141318	Batch Ref (VP)	987	141229	1205	151009	141352	141364	629	Batch Ref (VP)	301
D	1211	151217	131276	141202	151178	Batch Ref (VP)	592	Batch Ref (VP)	141346	1226	151019	301
E	1175	1111	1236	Batch Ref (VP)	534	141130	Batch Ref (VP)	141091	141348	141337	141362	141372
F	141365	Batch Ref (VP)	151187	1262	141440	141236	1189	1034	Batch Ref (VP)	638	141088	1132
G	532	1210	141387	141078	1171	Batch Ref (VP)	141217	Batch Ref (VP)	1018	1163	1237	141370
H	1133	151034	141099	151190	Batch Ref (VP)	1167	141353	1113	1078	141070	1229	Batch Ref (VP)

	n	
Batch Ref	8	Ventricular Pool
Healthy Control	51	Group 1
AD/MCI	14	Group 2
PD/PDI-MCI/LBD	15	Group 3
Batch QC	8	Golden West

Row 1		Row 2		Row 3		Row 4		Row 5		Row 6		Row 7		Row 8	
Batch Ref (VP)	B1VP-01	Batch QC (GW)	B1QC-02	687	ADC1-21	1211	ADC1-31	1175	ADC1-41	141365	ADC1-51	532	ADC1-61	1133	ADC1-71
141171	ADC1-01	1207	ADC1-11	141318	ADC1-22	151217	ADC1-32	1111	ADC1-42	Batch Ref (VP)	B1VP-06	1210	ADC1-62	151034	ADC1-72
141201	ADC1-02	141232	ADC1-12	Batch QC (GW)	B1QC-03	131276	ADC1-33	1236	ADC1-43	151187	ADC1-52	141387	ADC1-63	141099	ADC1-73
613	ADC1-03	587	ADC1-13	987	ADC1-23	141202	ADC1-34	Batch Ref (VP)	B1VP-05	1262	ADC1-53	141078	ADC1-64	151190	ADC1-74
151280	ADC1-04	141335	ADC1-14	141229	ADC1-24	151178	ADC1-35	534	ADC1-44	141440	ADC1-54	1171	ADC1-65	Batch QC (GW)	B1QC-08
141361	ADC1-05	1116	ADC1-15	1205	ADC1-25	Batch QC (GW)	B1QC-04	141130	ADC1-45	141236	ADC1-55	Batch Ref (VP)	B1VP-07	1167	ADC1-75
1231	ADC1-06	Batch Ref (VP)	B1VP-02	151009	ADC1-26	592	ADC1-36	Batch QC (GW)	B1QC-05	1189	ADC1-56	141217	ADC1-66	141353	ADC1-76
1095	ADC1-07	1030	ADC1-16	141352	ADC1-27	Batch Ref (VP)	B1VP-04	141091	ADC1-46	1034	ADC1-57	Batch QC (GW)	B1QC-07	1113	ADC1-77
141374	ADC1-08	1197	ADC1-17	141364	ADC1-28	141346	ADC1-37	141348	ADC1-47	Batch QC (GW)	B1QC-06	1018	ADC1-67	1078	ADC1-78
Batch QC (GW)	B1QC-01	151068	ADC1-18	629	ADC1-29	1226	ADC1-38	141337	ADC1-48	638	ADC1-58	1163	ADC1-68	141070	ADC1-79
141345	ADC1-09	1014	ADC1-19	Batch Ref (VP)	B1VP-03	151019	ADC1-39	141362	ADC1-49	141088	ADC1-59	1237	ADC1-69	1229	ADC1-80
1091	ADC1-10	141112	ADC1-20	481	ADC1-30	301	ADC1-40	141372	ADC1-50	1132	ADC1-60	141370	ADC1-70	Batch Ref (VP)	B1VP-08

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9:14 pm

MAKE SURE HEATING PLATES/BLOCKS ARE AT CORRECT TEMP

MATERIALS:

- Pooled ventricular CSF; 8 60 ul aliquots
- GW CSF; 3 250 ul aliquots
- BCA reagent; 22,000 ul total
 - 21,560 ul of reagent A
 - 440 ul of reagent B
- 1M iodoacetamide (IAA) (1 plate)
- 1M DL-dithiothreitol (DTT) (1 plate)
- 50 mM Tris, pH 8.5
 - 30 mL
 - 1.5 mL 50 1M Tris + 28.5 mL water; pH to 8.5
- 2% SDS
 - Dilute 20% SDS with Tris pH 8.5
 - 2,000 ul 20% SDS in 18,000 ul Tris
- Resyn Biosciences MagResyn Hydroxyl particles
- 100% Acetonitrile (ACN)
- 70% Ethanol
 - 200 mL
- 95% Acetonitrile
 - 300 mL
- 100 ng/ul enolase
 - 1,750 ul (0.2 ug/ul) aliquot in freezer 3
 - Thaw, add 1,600 ul of it to reservoir with 1,600 ul of lysis buffer
- 50 mM ammonium bicarbonate (ABC)
- Pierce Porcine trypsin; 3 100-ug vials
 - Add 2,000 ul of 50 mM ABC to each vial to get working concentration of 0.05 ug/ul

- HPLC grade 100% formic acid
- 500 fmol Pierce Retention Time Calibrant
 - Dilute 5 pmol PRTC (stock) to 500 fmol

Materials cont'd

- Labeled binding, wash, and elution plates
- Labeled reservoirs

METHODS:

Protein Assay

Thaw everything, and put on ice in order in 4 buckets

Perform BCA assay on the to determine protein concentration

200 ul of BCA reagent into each well of the assay plate

*3 rows per plate, then bring it to the 37 degree room, so 4 total plates

14 of 2% SDS into the 4 sample wells

Add 2 ul of sample to the wells

Add 16 ul of each standard to the wells

Pipette up and down to mix and heat for 30 min at 37 C

Use Varioskan flash to obtain photometric reading of the plates

Calculations

Whichever sample has the lowest concentration will be the baseline, so all other samples will be brought up to this volume with DPBS

So the amount of 2% SDS added to every sample will be the same

sample	conc	amt need for 25 ug	amt DPBS needed to bring to lowest conc vol	ul enolase	vol 2% SDS to add	1M IAA	1M DTT	vol for 70% final conc of ACN
1 Batch Ref (VP)	.896	27.9	6.39	8	34.29	3.06	1.6	
2 141171	.941	26.56	7.73	8	34.29	3.06	1.6	190 µl
3 141201	1.204	20.77	13.52	8	34.29	3.06	1.6	
4 613	.769	32.5	1.79	8	34.29	3.06	1.6	
5 151280	.769	32.5	1.79	8	34.29	3.06	1.6	
6 141361	.914	27.35	6.94	8	34.29	3.06	1.6	
7 1231	.91	27.49	6.8	8	34.29	3.06	1.6	
8 1095	.846	29.65	4.44	8	34.29	3.06	1.6	
9 141374	.819	30.52	3.77	8	34.29	3.06	1.6	
10 Batch QC (GW)	.95	26.31	7.58	8	34.29	3.06	1.6	
11 141345	.973	25.7	8.59	8	34.29	3.06	1.6	
12 1091	.878	28.48	5.94	8	34.29	3.06	1.6	
13 Batch QC (GW)	.86	29.08	5.21	8	34.29	3.06	1.6	
14 1207	.882	28.33	5.86	8	34.29	3.06	1.6	
15 141232	.887	28.19	6.1	8	34.29	3.06	1.6	
16 587	.869	28.78	5.57	8	34.29	3.06	1.6	
17 141335	.742	33.69	1.1	8	34.29	3.06	1.6	
18 1116	1.122	22.28	12.91	8	34.29	3.06	1.6	
19 Batch Ref (VP)	.946	25.44	7.55	8	34.29	3.06	1.6	
20 1030	.882	28.33	5.86	8	34.29	3.06	1.6	
21 1197	1.	25.	9.29	8	34.29	3.06	1.6	
22 151068	.937	26.89	7.6	8	34.29	3.06	1.6	
23 1014	.914	27.35	6.94	8	34.29	3.06	1.6	
24 141112	.869	28.78	5.57	8	34.29	3.06	1.6	
25 687	0.416	2.97	3.15	31.82	8	34.29	3.06	1.6
26 141318	.869	28.78	5.57	8	34.29	3.06	1.6	
27 Batch QC (GW)	1.054	23.11	18.58	8	34.29	3.06	1.6	
28 987	.991	25.43	9.16	8	34.29	3.06	1.6	
29 141229	.882	28.33	5.86	8	34.29	3.06	1.6	
30 1205	.864	28.93	5.36	8	34.29	3.06	1.6	
31 151009	.878	28.48	5.94	8	34.29	3.06	1.6	
32 141352	.742	33.69	1.1	8	34.29	3.06	1.6	
33 141364	.824	30.36	3.88	8	34.29	3.06	1.6	
34 629	.91	27.49	6.8	8	34.29	3.06	1.6	
35 Batch Ref (VP)	.919	27.22	7.17	8	34.29	3.06	1.6	
36 481	.792	31.57	2.22	8	34.29	3.06	1.6	
37 1211	.946	25.42	7.87	8	34.29	3.06	1.6	
38 151217	.907	27.57	6.72	8	34.29	3.06	1.6	
39 131276	1.132	22.38	12.21	8	34.29	3.06	1.6	
40 141202	.828	30.48	4.11	8	34.29	3.06	1.6	
41 151178	.824	30.36	3.93	8	34.29	3.06	1.6	
42 Batch QC (GW)	.892	28.02	6.27	8	34.29	3.06	1.6	
43 592	.926	26.98	7.3	8	34.29	3.06	1.6	
44 Batch Ref (VP)	.941	26.56	7.73	8	34.29	3.06	1.6	
45 141346	.961	26.12	8.27	8	34.29	3.06	1.6	

50	Batch QC (GW)	.892	28.02	6.27	8	34.29	3.06	1.6
51	592	.926	26.98	7.31	8	34.29	3.06	1.6
52	Batch Ref (VP)	.941	26.56	7.73	8	34.29	3.06	1.6
53	141346	.961	26.02	8.27	8	34.29	3.06	1.6
54	1226	.804	31.1	3.19	8	34.29	3.06	1.6
55	151019	.892	28.02	6.27	8	34.29	3.06	1.6
56	301	1.015	24.64	9.65	8	34.29	3.06	1.6
57	1175	.877	28.49	5.8	8	34.29	3.06	1.6
58	1111	.819	30.54	8.75	8	34.29	3.06	1.6
59	1236	.838	29.82	4.47	8	34.29	3.06	1.6
60	Batch Ref (VP)	.985	25.97	8.92	8	34.29	3.06	1.6
61	534	.755	33.12	1.17	8	34.29	3.06	1.6
62	141130	1.01	24.76	9.63	8	34.29	3.06	1.6
63	Batch QC (GW)	.877	28.49	5.8	8	34.29	3.06	1.6
64	141091	.819	30.54	8.75	8	34.29	3.06	1.6
65	141348	.838	29.82	4.47	8	34.29	3.06	1.6
66	141337	.863	28.98	8.11	8	34.29	3.06	1.6
67	141362	1.064	23.5	10.73	8	34.29	3.06	1.6
68	141372	.961	26.02	8.27	8	34.29	3.06	1.6
69	141365	.941	26.56	7.73	8	34.29	3.06	1.6
70	Batch Ref (VP)	1.157	21.41	12.68	8	34.29	3.06	1.6
71	151187	.907	27.37	6.72	8	34.29	3.06	1.6
72	1262	.843	29.85	4.44	8	34.29	3.06	1.6
73	141440	.99	25.23	9.74	8	34.29	3.06	1.6
74	141236	.917	27.21	7.12	8	34.29	3.06	1.6
75	1189	.799	31.23	3	8	34.29	3.06	1.6
76	1034	.833	30	4.29	8	34.29	3.06	1.6
77	Batch QC (GW)	.912	27.12	6.87	8	34.29	3.06	1.6
78	638	.892	28.02	6.27	8	34.29	3.06	1.6
79	141088	.912	27.12	6.87	8	34.29	3.06	1.6
80	1132	.961	26.02	8.27	8	34.29	3.06	1.6
81	532	.802	31.17	3.12	8	34.29	3.06	1.6
82	1210	.729	34.29		8	34.29	3.06	1.6
83	141387	.844	29.63	4.66	8	34.29	3.06	1.6
84	141078	.875	28.57	5.72	8	34.29	3.06	1.6
85	1171	.823	30.38	3.91	8	34.29	3.06	1.6
86	Batch Ref (VP)	.875	28.57	5.72	8	34.29	3.06	1.6
87	141217	.943	26.52	7.77	8	34.29	3.06	1.6
88	Batch QC (GW)	.88	28.4	5.85	8	34.29	3.06	1.6
89	1018	.786	31.79	2.5	8	34.29	3.06	1.6
90	1163	.828	30.19	4.1	8	34.29	3.06	1.6
91	1237	.974	25.87	8.62	8	34.29	3.06	1.6
92	141370	.891	28.07	6.22	8	34.29	3.06	1.6
93	1133	.833	30	4.29	8	34.29	3.06	1.6
94	151034	.932	26.92	7.47	8	34.29	3.06	1.6
95	141099	1.	25	9.29	8	34.29	3.06	1.6
96	151190	1.208	20.69	13.6	8	34.29	3.06	1.6
97	Batch QC (GW)	1.	25	9.29	8	34.29	3.06	1.6
98	1167	1.208	20.69	13.6	8	34.29	3.06	1.6
99	141353	.953	26.23	8.06	8	34.29	3.06	1.6
100	1113	.969	25.11	8.49	8	34.29	3.06	1.6
101	1078	.901	27.75	6.84	8	34.29	3.06	1.6
102	141070	.938	26.67	7.12	8	34.29	3.06	1.6
103	1229	1.083	23.08	11.21	8	34.29	3.06	1.6
104	Batch Ref (VP)	1.	25	9.29	8	34.29	3.06	1.6

Protein denaturation

To each well add a volume of 2% SDS equivalent to the volume of CSF added ✓
 Pipette up and down gently ✓
 Add DPBS to bring up samples to the volume equivalent to the most dilute sample ✓

Sample preparation

Based on the concentrations, pull 25 ug and add to plate ✓
 Add 8 ul of 100 ng/ul enolase ✓

Reduction and alkylation

Volumes of reducing and alkylating agents will be calculated after the sample and buffer volumes are determined (above) ✓
 Add 1.5 ul of 1M DTT to each well, pipette up and down gently ✓
 Heat the plate at 95C for 10 min

70% new final

$$(1.0)(v_1) = (81.38 + v_1)(0.7)$$

$$v_1 = 56.97 + 0.7v_1$$

$$0.3v_1 = 56.97$$

$$v_1 = 189.88$$

Allow to cool

Add 5.2 ~~5.2~~ ul of 1M IAA to each well

Pipette up and down gently

Incubate at room temperature in the dark for 30 minutes

Protein precipitation

Add 12.5 ul of the Hydroxyl beads (at their stock concentration of 20 ug/ul) to each well

Add 190 ul of 100% ACN to each well (70% final concentration) to precipitate proteins

Allow to sit for 10 minutes at room temperature

NOTE: You cannot freeze the samples at this point--the hydroxyl beads will rupture when frozen.

Wash and digestion plate preparation

Wash plates (deep well):

- Washes 1-3; 1 mL 95% ACN
- Washes 4-5; 1 mL EtOH

Digestion/elution plate (deep well):

- 100 ul 50 mM ABC + 50 ul of 0.05 ug/ul Pierce Porcine trypsin (1:10 enzyme:protein ratio)

Kingfisher automated clean-up and digestion

Turn on Kingfisher and bring up adapted Resyn protocol (see attached)

Make sure binding plate volume has been adjusted

Add plates to instrument as indicated by the program

Hit "run" *started @ 5:48 pm 4/29/2021*

The automated clean-up and digestion will take about 90 minutes.

Quenching the digestion

Add 5.4 ul of 100% formic acid to quench

Freeze (-80C) or dry down in speed vacuum

In my case, I transferred the samples from the 96 well plate and into 8 individual 1.5 mL tubes (NOT lo-bind)

200 ~~200~~ 200
1000 into each well
3000 into vort
combine