Ammonium sulphate precipitation assay

Purpose:

Concentrating protein sample and that protein still has function. We will use this sample to run enzyme assay to see if our enzyme has the ability to deink.

Material:

- ammonium sulphate
- protein concentration tube

Procedure:

1. Measure the volume of the enzyme medium. Refer to Table 1 to determine the amount of $(NH_4)_2SO_4$ to add to bring the medium to 30% saturation
2. Pour the medium into a centrifuge bottle or large beaker and add the appropriate amount of $(NH_4)_2SO_4$. Add the stir bar and stir in the cold room for 30 min.
3. Remove the stir bar and collect the precipitated proteins by centrifugation at 2 000 rcf for 30 min.
4. Discard the supernatant and resuspend the pellet with minimal volume of PBS.
5. Put 500 μl resuspend sample into protein concentration column.
6. Centrifuge at 14000g, RT for 20 minutes.
7. Discard flow through, flip the column and put in a new eppendorf.

Table 1 Final concentration of ammonium sulfate: percentage saturation at 0° C[a]

8. Percentage saturation at 0 °C

9.

Initial concentration of ammonium sulfate (percentage saturation at 0 °C)	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
	Solid ammonium sulfate (grams) to be added to 100 ml of solution																
0	10.6	13.4	16.4	19.4	22.6	25.8	29.1	32.6	36.1	39.8	43.6	47.6	51.6	55.9	60.3	65.0	69.7
5	7.9	10.8	13.7	16.6	19.7	22.9	26.2	29.6	33.1	36.8	40.5	44.4	48.4	52.6	57.0	61.5	66.2
10	5.3	8.1	10.9	13.9	16.9	20.0	23.3	26.6	30.1	33.7	37.4	41.2	45.2	49.3	53.6	58.1	62.7
15	2.6	5.4	8.2	11.1	14.1	17.2	20.4	23.7	27.1	30.6	34.3	38.1	42.0	46.0	50.3	54.7	59.2
20	0	2.7	5.5	8.3	11.3	14.3	17.5	20.7	24.1	27.6	31.2	349	38.7	42.7	46.9	51.2	55.7
25		0	2.7	5.6	8.4	11.5	14.6	17.6	21.1	24.5	28.0	31.7	35.5	39.5	43.6	47.8	52.2
30			0	2.8	5.6	8.6	11.7	14.8	18.1	21.4	24.9	28.5	32.3	36.2	40.2	44.5	48.8
35				0	2.8	5.7	8.7	11.8	15.1	18.4	21.8	25.4	29.1	32.9	36.9	41.0	45.3
40					0	2.9	5.8	8.9	12.0	15.3	18.7	22.2	25.8	29.6	33.5	37.6	41.8
45						0	2.9	5.9	9.0	12.3	15.6	19.0	22.6	26.3	30.2	34.2	38.3
50							0	3.0	6.0	9.2	12.5	15.9	19.4	23.0	26.3	30.8	34.8
55								0	3.0	6.1	9.3	12.7	16.1	19.7	23.5	27.3	31.3
60									0	3.1	6.2	9.5	12.9	16.4	20.1	23.9	27.6
65										0	3.1	6.3	9.7	13.2	16.8	20.5	24.4
70											0	3.2	6.5	9.9	13.4	17.1	20.9

75	0	3.2	6.6	10.1	13.7	17.4
80		0	3.3	6.7	10.3	13.9
85			0	3.4	6.8	10.5
90				0	3.4	7.0
95					0	3.5
100						0

[a]Adapted from Dawson RMC, Elliott DC, Elliott WH, and Jones KM (eds.) (1969) *Data for Biochemical Research*, 2nd edn. London: Oxford University Press.

TCA protein precipitation assay

Purpose:

Concentrating protein sample and run the SDS-PAGE to see if we have the right protein.

Materials:

- 100% TCA
- Acetone
- Tris-base
- 2X Protein sample buffer
- 2-mercaptoethanol

Procedures:

1. Place medium with enzyme into 50 ml centrifuge tube and add in 100% TCA, which is a 6% final solution.
2. Mix carefully and incubate on ice for 15 minutes.
3. Spin down for 10 min at 4°at 13,000 g and remove the supernatant.
4. Wash the pellet with 1 ml ice-cold acetone. This helps remove acids and salts.
5. Cut off the end of a pipette tip to make the opening larger, move the sample from centrifuge tube to microcentrifuge tube.
6. Spin down for 10 min at 4° at 13,000 g, remove the supernatant and set for 15 minutes to air dry the pellet.
7. If necessary (too much salt left) redo step 4 and 6.
8. Resuspend pellet in SDS-PAGE protein sample buffer. The TCA pellet can be difficult to resuspend, and it may be necessary to work the pellet into solution with a pipette tip.
9. If the protein sample buffer turns yellow, add 2M Tris-base that has not been adjust for PH, 1 μ l at a time, until it turns blue again. Be sure to add an equal amount of Tris-base to each sample as the extra salt can cause the samples to run differently on the SDS-PAGE gel.
10. Add in 2-mercaptoethanol, which is a 10% final solution.
11. Boil sample at 95° for 5 minutes.
12. Make sure your sample is still blue, if it is not, add in more Tris-base.
13. Ready for loading SDS-PAGE.

Small tips to rid of bubbles:

1. Spray ethanol in the air and that small amount of ethanol touch the bubbles, it will that bobbles be fragile and broken.
2. Centrifuge the sample in low speed for a small amount of time.

Enzyme condensation assay

Purpose:

To condense yeast secreted enzymes in the medium.

Material:

- Medium with yeast secreted enzymes(induced xylanase/ lipase; non-induced xylanase/ lipase)
- Amicon® Ultra-15 centrifugal filter
- 1X PBS
- Centrifuge tube

Procedure:

10. Use pipet aid to suck 12 mL medium and put into Amicon® Ultra-15 centrifugal filter
11. Centrifuge the medium at 4˚C, 5000g for 50 min
12. Discard the filtrate
13. Collect the protein solution remain on the filter
14. Add 150 μL 1X PBS and rinse the filter to collect the protein remain on it
15. Put all the collection in a bacteria free centrifuge tube
16. The condensed enzyme were used to do SDS PAGE to check enzyme exists and add into paper pulp to test deink efficiency