

**ULTROGEL® A – ULTROGEL® AcA****PRODUCT DESCRIPTION**

ULTROGEL® AcA and A are supports for gel filtration chromatography of biological macromolecules. They are composed of an homogeneous network of polyacrylamide and agarose in bead form.

Supplied as an aqueous suspension containing 2 mM citrate buffer, pH 6 and 0,02% sodium azide, these products should be stored at + 4°C and must never be frozen.

ULTROGEL® AcA ULTROGEL® A	Acrylamide (%)	Agarose (%)	Bead diameter (µm)	Linear fractionation range (daltons) *	Exclusion limit
AcA 202	20	2	60 – 140	1,000- 15,000	22,000
AcA 54	5	4	60 – 140	5,000- 70,000	90,000
AcA 44	4	4	60 – 140	10,000- 130,000	200,000
AcA 34	3	4	60 – 140	20,000- 350,000	750,000
AcA 22	2	2	60 – 140	100,000- 1,200,000	3,000,000
A 2	2	–	60 – 140	120,000-23,000,000	50,000,000
A 4	4	–	60 – 140	55,000- 9,000,000	20,000,000
A 6	6	–	60 – 140	25,000- 2,400,000	4,000,000

PREPARATION OF THE COLUMN AND SAMPLE APPLICATION

- With a spatula, transfer the required volume of ULTROGEL® AcA or A into a vacuum flask having a capacity of two column volumes. Add to the suspension a volume of chosen buffer of approximately 40% of the gel volume. Remove the dissolved gas under vacuum and gentle agitation. (Avoid magnetic stirring which may break the gel beads).
- Pour the ULTROGEL® AcA or A suspension into a chromatographic column following the recommended flow rates and advices indicated below.

ULTROGEL® Column dimensions (cm)	1.6 x 40		1.6 x 100		2.5 x 40		2.5 x 100	
	Pump cm/h	Pressure cm/H ₂ O	Pump cm/h	Pressure cm/H ₂ O	Pump cm/h	Pressure cm/H ₂ O	Pump cm/h	Pressure cm/H ₂ O
AcA 22	7	60- 90	3.5	40- 80	4.5	20- 50	3.5	15- 40
AcA 34	30	60- 90	10	80-120	15	40- 60	10	60- 80
AcA 44	45	100-120	25	100-120	30	100-120	18	80-100
AcA 54	45	100-120	25	100-120	30	100-120	18	80-100
AcA 202	25	100-120	10	100-120	15	100-120	10	80-100
A 2	7	60- 80	3.5	40- 60	4.5	20- 50	3.5	15- 40
A 4	25	60- 90	10	80-100	15	40- 60	10	60- 80
A 6	35	100-120	20	80-100	25	100-120	15	80-100

- Connect a syringe to the column outlet with a short length of silicone rubber tubing.
- Introduce a small volume (2 cm high) of degassed chromatographic buffer into the column.
- Draw the buffer out of the column into the syringe and check the elimination of air bubbles.
- Clamp the silicone rubber tubing and remove the syringe.

- Pour the degassed ULTROGEL® AcA or A suspension into the column. Keep the column outlet clamped for 2-3 minutes to allow the gel particles to settle, then release the clamp and allow the column to fill.
- Add degassed buffer so that the gel surface never gets dry.
- Filling is complete when a clear interface is apparent between the gel and the buffer.
- Completely fill the tubing with buffer, and introduce the upper column adapter, avoiding introduction of air bubbles.
- Never place the column in direct sunlight and avoid temperature changes.
- Wash and equilibrate the column with the desired buffer (two column volumes are necessary).
- Clamp the column inlet (silicone rubber tubing).
- Connect a peristaltic pump to the column outlet.
- The pump being stopped, introduce the upper tubing of the column into the sample solution. Remove any air bubble.
- Start the pump in order to introduce the sample solution into the column (the flow rate must be reduced during the sample application). The sample volume should be 0.5-4 % of the total gel bed volume.
- Stop the pump and connect the column upper tubing to a buffer reservoir.

RECOMMENDED FLOW RATES FOR MAXIMUM RESOLUTION

ULTROGEL®	Column dimensions (cm)		1.6 x 40		1.6 x 100		2.5 x 40		2.5 x 100	
	Pump cm/h	Pressure cm/H ₂ O	Pump cm/h	Pressure cm/H ₂ O	Pump cm/h	Pressure cm/H ₂ O	Pump cm/h	Pressure cm/H ₂ O	Pump cm/h	Pressure cm/H ₂ O
AcA 22	2-3	20-40	2-3	30-50	2-3	10-20	2-3	10-30		
AcA 34	3-5	5-15	3-5	35-40	3-5	10-20	3-5	10-30		
AcA 44	3-6	5-20	3-6	10-25	3-6	10-25	3-6	10-30		
AcA 54	3-6	5-15	3-6	10-25	3-6	10-30	3-6	10-30		
AcA 202	4-6	15-25	4-6	15-35	4-6	20-30	4-6	15-35		
A 2	3-5	8-12	3.5	17-25	3.5	10-14	3.5	12-20		
A 4	4-6	12-16	4-6	20-27	4-6	12-16	4-6	22-30		
A 6	4-6	7-11	4-6	13-20	4-6	8-12	4-6	19-28		

MAINTENANCE OF ULTROGEL® AcA OR A COLUMN

The best way of preserving ULTROGEL® AcA or A gel between experiments is to maintain a buffer running through the column at very low flow rate. A buffer containing 0.02% sodium azide would prevent any column degradation caused by microbial attack.

If it is not possible to keep the buffer flow, clamp the column inlet and outlet and store it at constant temperature. Before using the column again, check that the inlet and outlet tubings are thoroughly filled with buffer. If the column is stored for a long time, it is advisable to reequilibrate the gel in a fresh buffer solution before applying the sample.

ULTROGEL® AcA or A may also be taken from the column and stored in 2 mM citrate buffer, pH 6 containing 0.02% sodium azide. The volume of buffer used is about 50% of the gel volume.