

Recommendations for CHT™ Ceramic Hydroxyapatite Column Packing

CHT Column Packing — Scalable and Easy

CHT Ceramic Hydroxyapatite Media is incompressible and has high specific gravity, a rapid settling rate, and sensitivity to mechanical shear. These variations from traditional compressible resins have to be considered when designing CHT column-packing protocols. This wall chart lists some recommendations and guidelines for successful CHT Column packing. The packing protocol for CHT can be divided into five steps.

Step 1 Column Preparation

Recommended columns:

- Pilot- and small-scale manufacturing: Open columns
- Large-scale manufacturing: Closed columns

Frit/filter pore size:

- ≤10 μm for 40 μm CHT
- 20 μm for 80 μm CHT

Other considerations:

- Ensure that the column is as level as possible
- Ensure that the usable column height is at least twice the final bed height

Step 2 Slurry Preparation

Requirements

- CHT is provided as a dry powder. Wear proper protective equipment (PPE) when transferring the powder
- Prepare slurry directly in the column or in a slurry tank. Recommended buffers are shown in Table 1
- Calculate the amount of dry powder and buffer needed to make the required volume of slurry. For columns ≤20 cm, use 0.63 g powder/ml packed bed as the CHT density. For larger columns, use a value of 0.60 g/ml. CHT absorbs ~90% of its volume in buffer during the initial hydration. A sample calculation is shown below
 - To make 1 L of 50% slurry (500 ml packed bed)
 - Amount of powdered CHT required: 315 g (500 ml * 0.63 g/ml)
 - Amount of buffer required: 500 ml + 450 ml (90% of 500 ml)

Table 1. Recommended buffers for CHT Columns

	Hydrating	Equilibrating	Testing
20 mM NaPi, 150 mM NaCl pH 7.2–7.4 (PBS)	•	•	
200–400 mM NaPi, pH >7.2	•		
0.15–1 N NaOH	•		
20 mM NaPi, 0.6–1.2 M NaCl, pH 7.2–7.4			•
20 mM NaPi, 1–2% acetone, pH 7.2–7.4			•

Protocol

- Manual mixing: use a plastic paddle (Figure 1). Mix with J strokes or a side-to-side motion. Mixing in a circular manner may not achieve a uniform slurry
- Automated mixing: use a low shear hydrofoil impeller (Figure 2). Do not use impellers designed for buffer preparation
- Minimize the time that the CHT Media is mixed to prevent bead fracture
- Prepare slurries used in pack-in-place column systems as close to 30% as possible. For other column types, slurries of up to 50% can be used
- In-column preparation: with column top removed, pour buffer into column, add CHT, and stir manually. Avoid simple circular agitation to prevent mound formation



Fig. 1. (l) Polypropylene paddle for manual mixing.



Fig. 2. (r) Low-shear hydrofoil impeller for automated mixing.

Step 3 Slurry Transfer (30–50%)

- Manual: pour slurry into open column
- Pump: pump slurry into column. Use only a diaphragm pump to prevent bead damage and avoid creation of fines. Do not use peristaltic or rotary lobe systems
- Pressure: use a pressurized slurry vessel
- Syringe/vacuum: use suction with a column top (avoid introducing air bubbles). Visit bio-rad.com/CHTSyringePacking to watch the complete packing procedure

Step 4 Column Packing

Tips

- Allow the slurry to settle until a 2–3 cm clear supernatant layer forms (Figure 3) before putting in the piston
- Confirm that the column is purged of air before continuing
- Pack at 150–300 cm/hr with flow and/or piston movement until the bed is fully consolidated. Since CHT is incompressible, the bed will not rebound
- Leave a gap of 1–5 mm between the headplate and the bed (Figure 4) to avoid crushing particles

Methods

Recommended types of packing include flow, axial, hybrid (axial + flow), and syringe packing.

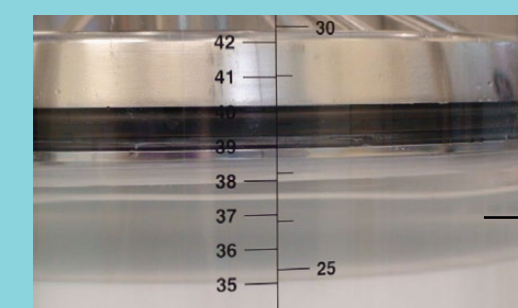


Fig. 3. Settled slurry.

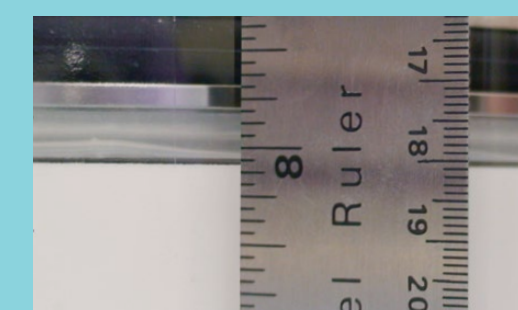
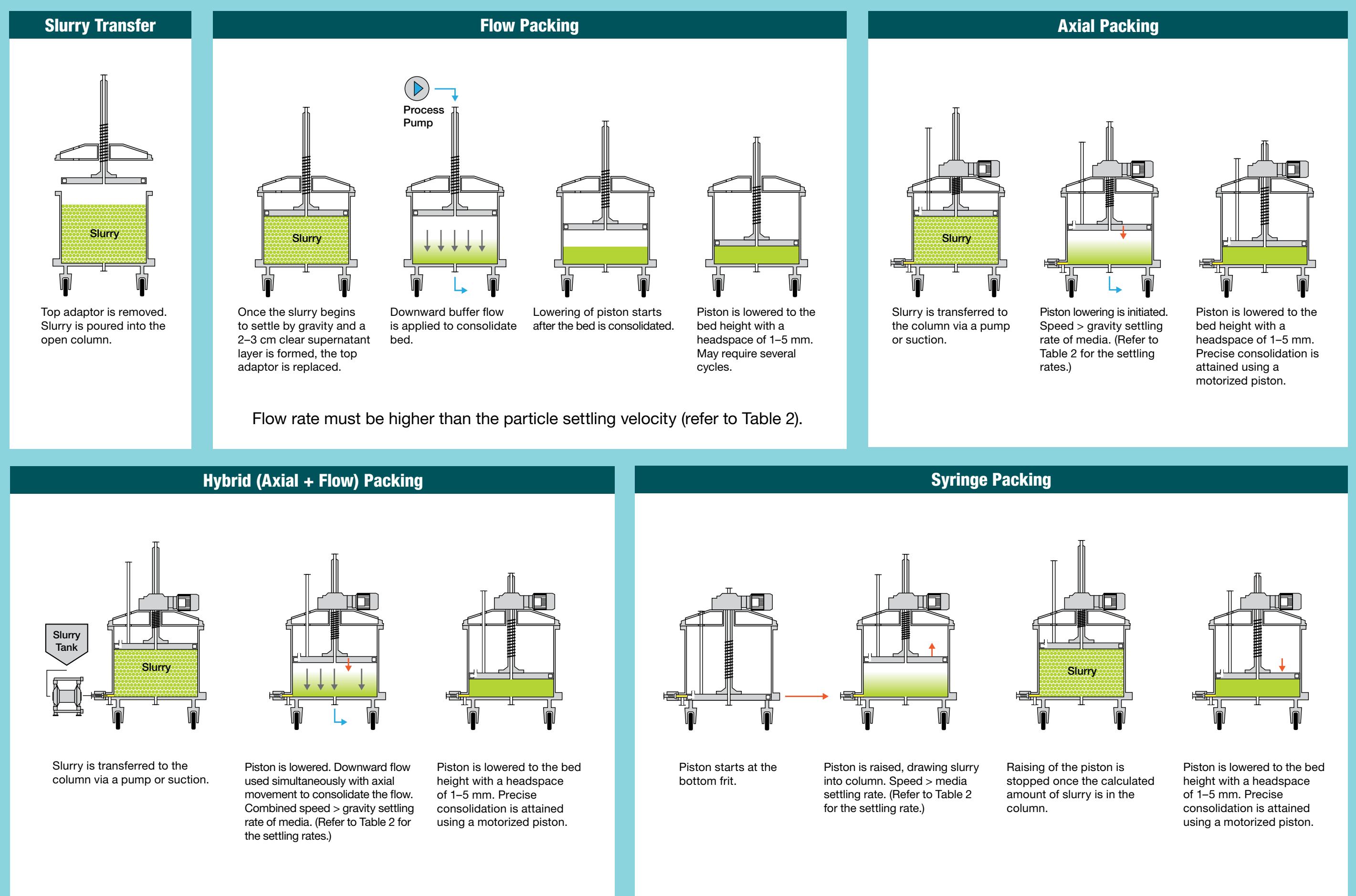


Fig. 4. Headspace of 1–5 mm between headplate and the bed.



Step 5 Packed Column Evaluation/Qualification

Evaluation carried out by:

- Asymmetry (As)/HETP testing by conductivity
 - Equilibrate with phosphate buffered saline (PBS) or similar buffer
 - Inject with PBS + 1 M NaCl (1–2% of bed volume)
 - Always use the same level of phosphate in the equilibration and injection buffers
- Alternate test methods
 - UV (acetone, vitamin B₁₂)
 - Alternate conductivity with 1 N NaOH tracer in 0.1 N NaOH

Flow rate, testing tracer concentration and volume, column types, and external system volume can all impact qualification result values.

A well-packed column will often produce two peaks. A small second peak is seen with NaCl tracers. This is due to an interaction between the background phosphate and the column and is normal. The second peak is not seen with acetone or NaOH.

CHT Packing — Things to Remember

Table 2. Recommendations

Recommended slurry concentration	30–50% v/v
Tapped settled density	0.63 g/ml (kg/l); 0.60 g/ml (kg/l) for columns >20 cm in diameter
Filter (frit) pore size	≤10 μm for 40 μm CHT; 20 μm for 80 μm CHT
Settling rate	40 μm: 35–125 cm/hr; 80 μm: 125–275 cm/hr

Post-Column Packing Considerations

- CHT beds can continue to consolidate after initial packing, as also seen with silica and controlled-pore glass
- Bed compaction and an apparent loss in bed height (appearance of headspace) can occur over many cycles or if the column is moved from one room to another
- If necessary, lower the flow adapter further to minimize the additional headspace
- CHT binds metals that are in process solutions, which may cause discoloration at the top of the column. Consult your process specialist for mitigating strategies
- The lifetime of CHT can be enhanced through a variety of methods. Consult your process specialist for further details

CHT Column Packing



Do's

- Ensure column leveling
- Use plastic paddles for manual mixing and low-shear hydrofoil impeller for automated mixing
- Use only a diaphragm pump for media transfer
- Leave a headspace of 1–5 mm
- Restrict settling time to <10 min for optimal packing



Don'ts

- Do not compress
- Defining or decanting steps are not required
- Avoid mechanical shear and compression
- Don't perform buffer upflow with packed CHT (except during unpacking)

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Additional Support

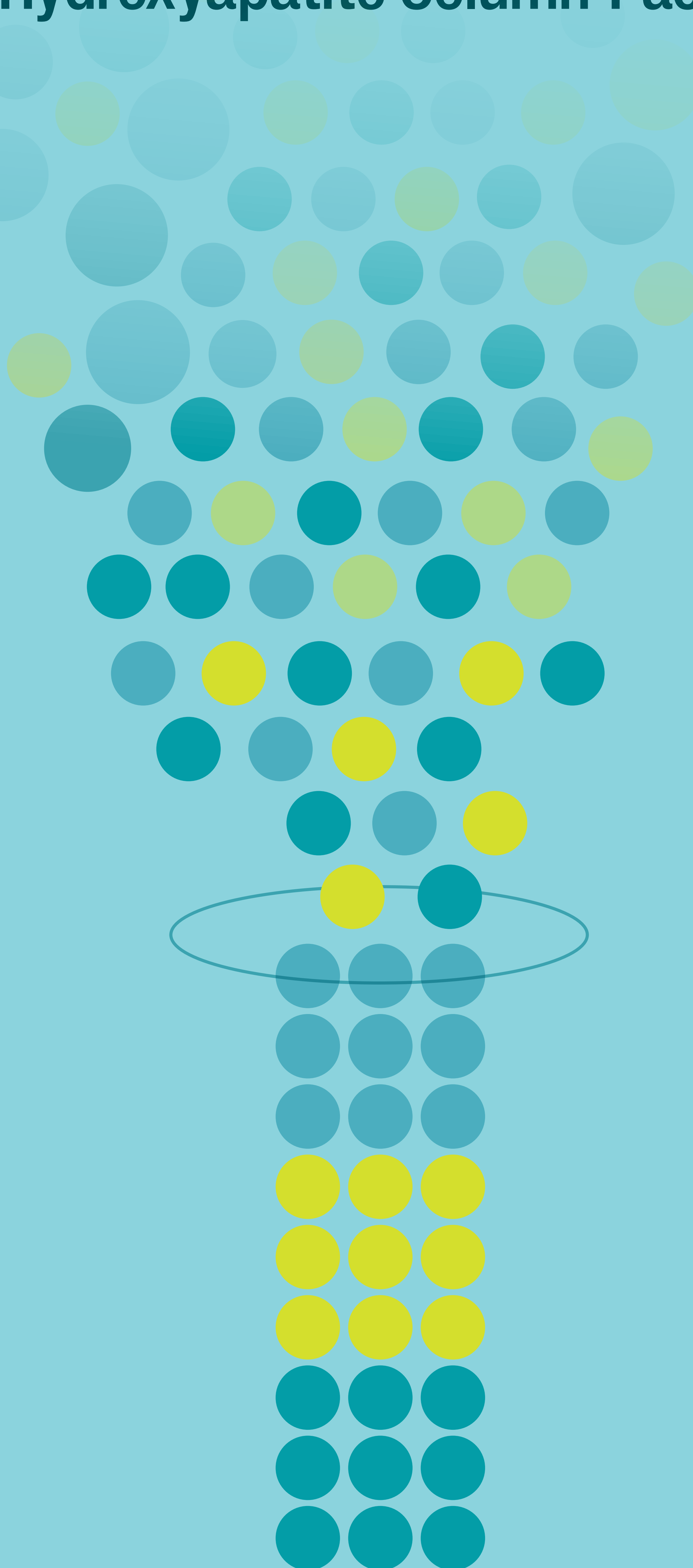
Visit bio-rad.com/ProcessResins to get detailed information about CHT Ceramic Hydroxyapatite and bio-rad.com/ProteinPurification to learn about its various applications.

Refer to bulletin 6086 for complete details on how best to use CHT Media.

Visit bio-rad.com/CHTVideoLibrary to watch CHT column-packing videos.

Contact us at process@bio-rad.com for column packing support or to use our process chromatography application laboratory services.

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