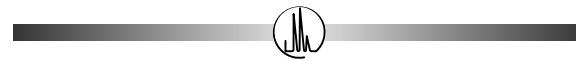

Chelex® 100 and Chelex 20 Chelating Ion Exchange Resin

Instruction Manual

BIO-RAD

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547

LIT200 Rev B

Introduction

Chelex chelating ion exchange resin has unusually high preference for copper, iron, and other heavy metals over monovalent cations such as sodium and potassium. Its selectivity for divalent over monovalent ions is approximately 5,000 to 1, and it has a very strong attraction for transition metals, even in highly concentrated salt solution.

Technical Description

Chelating resin is available as Analytical Grade Chelex 100 resin, Biotechnology Grade Chelex 100 resin, and Technical Grade Chelex 20 resin. The Analytical Grade Chelex 100 resin has been exhaustively sized, purified, and converted to make it suitable for accurate, reproducible analytical techniques. Biotechnology Grade Chelex 100 resin is analytical grade resin which is certified to contain less than 100 micro-organisms per gram of resin. Technical Grade Chelex 20 resin is coarse mesh resin useful for large

scale clean-up, for example metals from waste waters, where analytical purity is not a major concern.

Chelex 100 resin and Chelex 20 resin are styrene divinylbenzene copolymers containing paired iminodiacetate ions which act as chelating groups in binding polyvalent metal ions. Chelex chelating resin is classed with the weakly acidic cation exchange resins by virtue of its carboxylic acid groups, but it differs from ordinary exchangers because of its high selectivity for metal ions and its much higher bond strength.

Chelex chelating resin is efficiently regenerated in dilute acid and operates in basic, neutral, and weakly acidic solutions of pH 4 or higher. At very low pH, the resin acts as an anion exchanger. Figure 1 shows the zwitterionic forms of the Chelex resin as a function of pH.

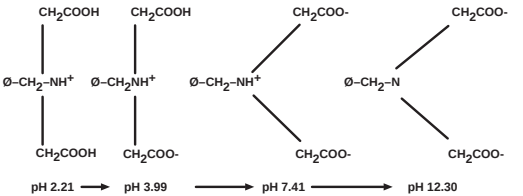

Fig. 1. Change in structure of Chelex resin with increasing pH.

Selectivity for Heavy Metal Ions

The selectivity of Chelex resin for metal cations corresponds to that of iminodiacetic acid. A list of selectivity factors for several divalent cations is given in Table 1. The selectivity factor is a quantitative measure of the affinity that Chelex resin displays for a particular cation compared to its affinity for a reference cation, in this case Zn^{+2}.

Table 1. Selectivity for Divalent Cations

Hg^{+2}	1060	Fe^{+2}	0.130
Cu^{+2}	126	Mn^{+2}	0.024
UO^{+2}	5.70	Ba^{+2}	0.016
Ni^{+2}	4.40	Ca^{+2}	0.013
Pb^{+2}	3.88	Sr^{+2}	0.013
Zn^{+2}	1.00	Mg^{+2}	0.009
Co^{+2}	0.615	Na^{+1}	0.0000001
Cd^{+2}	0.390		

Actual selectivity values for any particular system depend on the pH, ionic strength, and the presence of other complex-forming species. Thus Hg^{+2} appears high in the selectivity series in the presence of nitrate ions, but low in the series in the presence of chloride ions, with which it forms a complex. The approximate order of selectivity for cations in nitrate or chloride solutions is:

Cu^{+2}>>Pb^{+2}>Fe^{+3}>Al^{+3}>Cr^{+3}>Ni^{+2}>Zn^{+2}>Ag^{+} >Co^{+2}>Cd^{+2}>Fe^{+2}>Mn^{+2}>Ba^{+2}>Ca^{+2}>>>Na^{+}

A selectivity series for cations in an acetate buffer system at pH 5 is:

Pd^{+2}>Cu^{+2}>>Fe^{+2}>Ni^{+2}>Pb^{+2}>Mn^{+2}>>

Ca^{+2} = Mg^{+2} >>> Na^{+}

The selectivity for various cations in aqueous solutions at pH 4 is:

Hg^{+2}>Cu^{+2}>Pb^{+2}>>>Ni^{+2}>Zn^{+2}>Cd^{+2}>Co^{+2}>Fe^{+2}> Mn^{+2}>Ca^{+2}>>>Na^{+}

The selectivity at pH 9 in the presence of 1.5 M $(NH_4)_2SO_4$ is:

Co^{+2}>Ni^{+2}>Cd^{+2}>Cu^{+2}>Zn^{+2}>Ca^{+2}>>>Na^{+}

Instructions for Use

Chelex resins may be used with either a batch method or a column method.

Batch Method

The batch method is the addition of resin directly into the sample followed by stirring.

1. Weigh out about 5 grams of resin for every 100 ml of sample. For larger scale applications or when a more exact amount of resin is needed, use the capacity guidelines given below to calculate the resin volume for the specific sample metal concentration.
2. Add resin to the sample and stir or shake (gently) for 1 hour.
3. Filter or decant the sample from the resin.

Column Method

The column method involves pouring a column with the Chelex resin and passing the sample through

to achieve the separation. Although large mesh material (50-100 mesh) allows rapid flow rates and the ability to process large volumes of solution, resolution may be sacrificed. On the other hand, small mesh material (200-400 mesh) can achieve very high resolution and analytical results, but will require longer process time due to the slow flow rate.

1. Calculate the amount of resin required based on the expected metal concentration. If the metal concentration is unknown, begin with 5 grams of resin for 100 ml of sample, and then optimize the volumes after obtaining the results.
2. Prepare a buffer with a pH and ionic concentration that will allow the metal to be ion-exchanged easily onto the column. Use the information from Table 1, and the selectivity comparisons of different pH solutions mentioned above, to optimize the buffer. For unknown solutions, use deionized water.
3. Slurry the resin in the buffer, and pour the column. Allow several bed volumes to pass through the column to insure a well packed bed.

4. Slowly add the sample to the column, taking care not to disturb the resin bed.
5. Initiate flow. Discard initial buffer from the void volume.
6. If a metal free solution is the goal, collect effluent. If the concentrated metals are of interest, allow all of the sample to pass through the column, then elute the metals off the resin with a solution containing a counterion of higher selectivity than the bound metal.

Ion Exchange

The quantity of cations exchanged is a function of pH. Exchange is very low below pH 2, increases sharply from pH 2 to 4, and reaches a maximum above pH 4. Any metal removed from solution is replaced by an equivalent amount of the ions originally on the resin. Usually an alkali metal form is best. The resin is supplied in the sodium form, but may be used in the potassium or ammonium form as well. These weakly held ions allow other ions to be readily adsorbed. The

capacity of the sodium form of the resin is 0.4 meq/ml (defined as $Cu(NH_3)_4^{+2}$ uptake).

Elution, Regeneration, and Conversion

Metals can be removed with Chelex chelating resin using either the batch or the column technique, though the column technique is generally more efficient. The most effective agents to elute the metals from the resin are acids. Concentrated salt solutions are often useful for selective elution, but are generally inefficient in removing strongly absorbed metals.

Regeneration of the resin to a salt form is a two-step process. The resin is first converted to the hydrogen form using acid, then converted to the desired ionic form using the hydroxide of the cation desired. The regeneration to the sodium form of resin loaded with copper would proceed as follows:

$$\textbf{Resin-Cu} + 2\,\textbf{HCl} \rightarrow \textbf{Resin-H} + \textbf{CuCl}_2$$

$$\textbf{Resin-H} + \textbf{NaOH} \rightarrow \textbf{Resin-Na} + \textbf{H}_2\textbf{O}$$

The following sequence should be used: 2 bed volumes in 1 N HCl, 5 bed volumes water rinse, 2 bed

volumes 1 N NaOH, 5 bed volumes water rinse. In some cases, especially with strongly complexed metals such as iron, complete regeneration can be accomplished only by using the two-step regeneration procedure. Single-step conversions are adequate when going from weakly held to strongly held ions. Thus the calcium form is prepared from the sodium form using 2 bed volumes of 2 N calcium chloride.

Chelex 100 resin undergoes volume changes when its ionic form is altered or the external medium is changed. The resin swells 100% in going from the hydrogen to a monovalent salt form. Therefore, normal precautions, such as wrapping columns with tape, should be taken to protect against glass breakage. Using the resin in the calcium form is a method that has been used to prevent shrinkage upon elution. The resin volume in water of different ionic forms is:

Na^{+} 1.00; H^{+} 0.45; Cu^{+2} 0.60; Fe^{+2} 0.45; Zn^{+2} 0.55; Ca^{+2} 0.53; K^{+} 1.06; Li^{+} 0.98; Ag^{+} 0.70; Cr^{+3} 0.53.

The resin volume of the sodium form in various solvents is (the resin exhibits appreciable capacity in organic solvents):

water 1.00, acetone 0.47, methanol 0.70, ethanol 0.45, isopropanol 0.48, ethyl acetate 0.96.

Calculating Capacity

A step-by-step method is used for determining the approximate amount of resin needed to remove heavy metal ions from aqueous systems.

1. Determine total volume of solution to be treated to remove heavy metals.

   Example: 10 liters

2. Calculate the average molecular weight of metals to be exchanged.

$$\frac{\sum \text{MW}}{\text{n}} = \text{average MW}$$

Example: Using Cu^{+2}, Cd^{+2}, Cr^{+3};
227.9/3=76 grams/mol

3. Calculate the total weight of the metals. Usually, metals are measured in parts per million (ppm). In aqueous solutions, ppm can be assumed to be mg/liter.

Example: Cu^{+2} = 3 ppm
Cd^{+2} = 5 ppm
Cr^{+3} = 2 ppm
10 ppm or 10 mg/liter
10 mg/liter x 10 liters = 100 mg

4. Convert the weight determined in step 3 to equivalence. Equivalence = weight in grams/equivalent weight, where equivalent weight = molecular weight/average valence.

Example: $$\frac{100 \text{ mg}}{\frac{76 \text{ g/mol}}{2 \text{ eq/mol}}} = 2.63 \text{ meq}$$

5. The wet capacity of Chelex resin is 0.40 meq/ml. Knowing this, the volume of resin needed can be calculated.

Example: $$\frac{2.63 \text{ meq}}{0.4 \text{ meq/ml}} = 6.58 \text{ ml}$$

6. Convert the volume in step 5 to weight. The density of Chelex resin is 0.65 g/ml.

Example: 6.58 ml x 0.65 g/ml = 4.3 grams

pH Stability

Chelex resin is stable over the entire pH range and functionally active from pH 2-14.

Flow Rates

If a tightly held cation is to be isolated from a solution of weakly held cations, a flow rate in excess of 20 cm/min can often be used. Separations between similar species and efficient regeneration and conversion require lower flow rates, usually less than 4 cm/min.

Buffering

Chelex resin in the hydrogen form has a pH of 2–3. The pH in the sodium form is about 11 and may be slowly lowered by extended water washing. However, a more satisfactory procedure for adjusting the pH is to use a buffer. Thus, a sodium form at pH 6.3 can be prepared by rinsing with 4 bed volumes of 0.5 M sodium acetate buffer, followed by 5 bed volumes of water.

Storage

Chelex resin is stable for at least 2 years when stored sealed in the original container at 22 °C. It should be stored in a salt form such as sodium or ammonium. If left in the hydrogen form for more than a few hours, the resin has a tendency to lose chelating capacity. Should such a loss occur, the resin can be regenerated by heating it at 60 °C in 30-50% alkali for 24 hours. Free iminodiacetic acid produced upon long standing (detected by its odor) may be extracted by methanol or by heating to

80 °C in 3 N ammonium hydroxide for 2 hours. The resin is autoclavable in the sodium form.

Applications of Chelex Resins

Chelex 100 resin has found many uses. These include analysis of trace metals in natural waters, reagents, biochemical, and physiological fluids; removal of trace metals from reagents, biochemicals, physiological fluids, culture media, soils, and enzyme systems; recovery of metals from process streams; and chromatography of closely related metals.

Trace Metal Removal

Chelex resin offers a rapid and thorough method for removing trace metal contaminants that could have an effect on biological fluids or biological systems under study. A unique ion exchange resin that is more selective for multivalent metals than the standard cation exchange resins, Chelex resin will scavenge multivalent metal ion contaminants without altering the concentration of nonmetallic ions. In most cases,

neither column nor batch treatment with Chelex 100 resin has any effect on protein concentration or enzyme activity. Where low protein recovery is a problem, the protein can be dialyzed against a buffer containing Chelex 100 resin. Table 2 gives examples of trace metal removal with Chelex 100 resin.

Glyphosate Concentration with Chelex 100 Resin

Chelex 100 resin may be used to concentrate glyphosate [N-(phosphonomethyl) glycine] when the resin is in the iron form. When environmental water or an aqueous extract of crops such as soybeans, grapes, cabbage, or alfalfa is applied to Chelex 100 resin (100-200 mesh, iron form), glyphosate complexes with the iron form Chelex 100 resin. The glyphosate may subsequently be eluted with 6 M HCl and applied to AG® 1-X8 resin (200-400 mesh, chloride form) for anion exchange cleanup. The glyphosate along with AMPA (aminomethyl phosphonic acid), its breakdown product, may then be quantified with the Aminex® glyphosate analysis column.

Metal Analysis with Chelex Resin

Many methods of metal analysis, such as neutron activation and atomic absorption, depend on the prior separation and concentration of the metals from such samples as air, soil, industrial waste waters, and biological extracts. Trace metals can be concentrated by adsorption to Chelex chelating resin. The use of Chelex resin to preconcentrate samples for analysis has been extensively reviewed.[1] Determination of subnanogram levels of Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn can be achieved by using Chelex 100 resin 200-400 mesh, with graphite furnace atomic adsorption spectrometry.[2] Chelex 100 resin is also effective in concentrating traces of Cd, Co, Cu, Mn, Ni, Pb, and Zn from various food digests. Average recoveries were 98.1% determined with standards.[3]

Table 2. Sample Applications with Chelex 100 Resin

Application	Reference
Preparation of metal-free apo-enzyme	O'Keefe, E. T., Hill, R. L. and Bell, J. E., *Biochem.*, **19,** 4954 (1980).
Removal of calcium from sarcoplasmic reticulum vesicles	Chiesi, M. and Inesi, G., *Biochem.* **19,** 2912 (1980).
Removal of extraneously bound metal ions from enzymes prior to NMR and ESR studies	Barker, R., et al., *Biochem. J.*, **177,** 289 (1979).
Batch removal of calcium from whole blood	Raymond, F A. and Weinshilboum, R. M., *Clin. Chim. Acta*, **58**, 185 (1975).
Removal of metals from ATP	Sontheimer, G. M., et al., *Biochem.*, **26,** 2701 (1987).
Removal of metals from buffer, brine, and biological solutions	Knapp, G., et al., *J. Anal. Atomic Spectrometry*, **2,** 611 (1987); Laue, T. M., et al., *Biochem.*, **28,** 4762 (1989).
Removal of metals from S100b and melittin	Baudier, J., et al., *Biochem.*, **26,** 2886 (1987).
Purification of dinucleotides	Reinhardt, C. G. and Krugh, T. R., *Biochem.*, **17,** 4845 (1978).

Application	Reference
Removal of metals from cell suspension	Bosron, W. F., Kennedy F. S. and Vallee, B. L., *Biochem.*, **14**, 2275 (1975).
Batch removal of metals from urine	Agarwal, M., Bennett, R. B., Stump, I. G. and D'Avria, J. M., *Anal. Chem.*, **47,** 924 (1975).
Removal of metals from enzyme solutions	Dunn, M. F., Pattison, S. E., Storm, M. C. and Quiel, E., *Biochem.*, **19,** 718 (1980).
Removal of metals from guinea pig complement	Amiraian, K., McKinney, J. A. and Duchna, L., *Immunology*, **26,** 1135 (1974).
Removal of aldehydes and peroxides from polyethylene glycol	Ray, W. J. and Pavathingal, J. M., *Anal. Biochem.*, **146,** 307 (1985).
Removal of interfering components from myo-inositol	Brandt, S. J., Dougherty, R. W., Lapetina, E . G. and Niedel, J. E., *Proc. Nat. Acad. Sci.*, **82,** 3277 (1985).
Calcium removal from protein kinases	Putnam-Evans, C. L., Harmon, A. C. and Cormier, M. J., *Biochem.*, **29,** 2488 (1990).
Removal of divalent cations from NMR stock solutions	Devlin, C. C. and Grisham, C. M., *Biochem.*, **29,** 6192 (1990). Chuknyisky, P. P., Rifkind, J. M., Tarien, E., Beal, R. B. and Eichhorn, G. L., *Biochem.*, **29,** 5987 (1990).

Application	Reference
Removal of paramagnetic impurities from oligonucleotides for NMR analysis	Kochoyan, M., Leroy, J. L. and Guéron, M., *Biochem.*, **29,** 4799 (1990). Schroeder, S. A., Roongta, V., Fu, J. M., Jones, C. R. and Gorenstein, D. G., *Biochem.*, **28,** 8292 (1989).
Trace metal removal from buffer solutions and seawater	Pai, S.-C., Chen, T.-C., Wong, G. T. F. and Hung, C.-C., *Anal. Chem.*, **62,** 774 (1990).
Iron removal from bacteria	Trost, J. T. and Blankenship, R. E., *Biochem.*, **28,** 9898 (1989).
Trace metal ion removal from buffer solutions	Yong, G., Leone, C. and Strothkamp, K. G., *Biochem.*, **29,** 9684 (1990). Severns, J. C. and McMillin, D. R., *Biochem.*, **29,** 8592 (1990) Shang, Z., Liao, Y.-D., Wu, F. Y.-H. and Wu, C.-W., *Biochem.*, **28,** 9790 (1989). Chung, H. K. and Ingle, J. D., *Anal. Chem.*, **62,** 2547 (1990). Grimshaw, C. E., Shahbaz, M. and Putney, C. G., *Biochem.*, **29,** 9936 (1990).
Removal of multivalent cation contaminants	Swanson, J. E. and Feigenson, G. W., *Biochem.*, **29,** 8291 (1990).

Application	Reference
Purification of NMR reagents	Brito, R. M. M., Rudolph, F.B. and Rosevear, P. R., *Biochem.*, **30,** 1461 (1991). Ray, W. J., Burgner, J. W. and Post, C. B., *Biochem.*, **29,** 2770 (1990).
Calcium removal from calmodulin	Vorherr, T., James, P., Krebs, J., Enyedi, A., McCormick, D. J., Penniston, J. T. and Carafoli, E., *Biochem.*, **29,** 355 (1990).
Purification of zinc isotopes from human blood and excrement	Gökmen, I. G., Aras, N. K., Gordon, G. E., Wastney, M. E. and Henkin, R. I., *Anal. Chem.*, **61,** 2757 (1989).
Calcium removal from buffer solutions	Kinoshita, C.M., Ying, S.-C., Hugli, T.E., Siegel, J. N., Potempa, L. A., Jiang, H., Houghten, R. A. and Gewurz, H., *Biochem.*, **28,** 9840 (1989). Thielens, N. M., Dorsselaer, A. V., Gagnon, J. and Arlaud, G. J., *Biochem.*, **29,** 3570 (1990).
Removal of zinc from buffer solutions	Jefferson, J. R., Hunt, J. B. and Ginsburg, A., *Biochem.*, **29,** 6687 (1990).
DNA extraction for PCR	Walsh, P. S., Metzger, D. A. and Higuchi, R., *BioTechniques*, **10,** 506 (1991).

Table 3. Other Examples of Metal Preconcentration and Separation on Chelex 100 Resin

Metal	Sample	Reference
As (V), As (III).	Industrial solutions	Chandra, M., et al., *Reactive Polymers*, **8,** 85 (1988).
Cu, Cd, Mn, Zn, Pb	River water	Liu, Y. and Ingle, Jr., J. D., *Anal. Chem.*, **61,** 525 (1989).
Cd, Cu, Pb, Zn	Environmental samples	Figura, P. and McDuffie, B., *Anal. Chem.*, **52,** 1433 (1980).
Uranium	Natural waters	Pakains, P., *Anal. Chim. Acta*, **120,** 289 (1980).
Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Sc, Sn,Th, U, V, Zn	Natural waters	Kingston, H. M. and R. R., *Anal. Chem.*, **55,** 1160 (1983); Greenberg, R. R. and Kingston, H. M., *J. Radioanal. Chem.*, **71,** 147 (1982).
Cd, Co, Cu, Fe, Mn, Ni, Zn	Seawater	Kingston, H. M., Barnes, I. L., Brady, T. J. and Rains, T. C., Pb, *Anal. Chem.*, **50,** 2064 (1978).
Cd, Zn, Pb, Fe, Mn, Cu, Ni, Co, Cr	Seawater	Sturgeon, R. E., Berman, S. S., Desaulniers, J. A. H., Mykytiuk, A. P, McLaren, J. W. and Russell, D. S., *Anal. Chem.*, **52,** 1585 (1980).

Metal	Sample	Reference
Fe, Cd, Zn, Cu, Ni, Pb, U, Co	Seawater	Mykytiuk, A. P., Russell, D. S. and Sturgeon, R. E., *Anal. Chem.*, **52,** 1281 (1980).
Cd, Pb, Ni, Cu, Zn	Seawater	Rasmussen, L., *Anal. Chim. Acta*, **125,** 117 (1961).
Cd, Ce, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sc, Sn, Th, U, Zn	Seawater	Kingston, H. M. and Greenberg, R. R., *Environ. Inter.*, **10,** 153 (1984).
Fe, Mn, Cu, Ni, Cd, Pb, Zn	Seawater	Paulson, A. J., *Anal. Chem.*, **58,** 183 (1986).
Fe, Zn, Mn	Biological materials	Pella, P. A., Kingston, H. M. and Sieber, J . R., *Anal. Chem.*, **55,** 1193 (1983).
V	Biological materials	Fassett, J. D. and Kingston, H. M., *Anal. Chem.*, **57,** 2474 (1985).
V	Seawater	Dupont, V., Auger, Y., Jeandel, C. and Wartel, M., *Anal. Chem.*, **63,** 520 (1991).

Ordering Information

Catalog Number	Product Description	Pkg. Size
142-2822	**Analytical Grade Chelex 100 Resin,** 50-100 mesh, sodium form	500 g
142-2832	**Analytical Grade Chelex 100 Resin,** 100-200 mesh, sodium form	500 g
142-2825	**Analytical Grade Chelex 100 Resin,** 100-200 mesh, iron form	100 g
142-2842	**Analytical Grade Chelex 100 Resin,** 200-400 mesh, sodium form	500 g
143-2832	**Biotechnology Grade Chelex 100 Resin,** 100-200 mesh, sodium form	100 g
745-7001	**Technical Grade Chelex 20 Resin,** 20-50 mesh, sodium form	10 kg