Seplife® Oligo dT(20)

1. Description

Seplife® Oligo dT(20) is an affinity chromatographic resin specifically designed for the capture of messenger RNA (mRNA) from complex matrixes, increasing its purity. The highly crossed linked styrene/divinylbenzene (DVB) matrix provides a rigid porous matrix which, following its coating with a hydrophilic layer, is functionalized with poly(DT) groups.

The role of the hydrophilic resin coating is to minimize non-specific interactions while the functional groups poly(DT) are specifically interacting with the polyA tail of the mRNA through base pairing.

Seplife® Oligo dT(20) has the following properties:

- Uniform particle size distribution of 50microns.
- High loading capacity
- High salt and pressure resistance
- Wide pH range stability
- Stable in a broad range of temperatures
- Provides efficient capture and release of the mRNA under standard purification conditions

Seplife® Oligo dT(20) is an affinity chromatographic resin with average particle size of 50 micron designed for the efficient capture of mRNA (up to 9000nt) from complex mixtures, with purity up to 98%.

2. Properties

Product	Seplife® Oligo dT(20)
Appearance	White spherical beads
Type	Affinity with dT(20) functional groups
Matrix	Polystyrene/DVB
Ligand density (μmol/ml)	0.25-0.35
Average particle size (μm)	50
pH stability	2-13
Chemical Stability	Stable in common aqueous salt solutions up to 5M NaCl. Stable in 20% and 70% ethanol, 20% acetonitrile and 30% isopropanol aqueous solutions at room temperature CIP recommended in 0.1M NaOH
Flow rate (cm/h)	> 400 cm/h, 0.3MPa (0.1M NaCl)
Recommended operating temperature (ºC)	2 - 65
Shipped as	Slurry in 20% ethanol solution

3. Instructions for use

3.1 Column packing

Column packing should be done according to standard operating procedures. It is important to ensure that each material is at its working temperature. Seplife® Oligo dT(20) resin is supplied in a slurry in 20% ethanol. In order to determine the necessary volume of resin to pack a defined column, it is necessary to determine or consider the slurry concentration and the compression factor. Seplife® Oligo dT(20) resin has a compression factor of 1.12; compression factor is determined as the ratio of gravity settled volume to packed bed volume.

Two column packing procedures may be applied: Constant pressure packing and Constant flow packing.

3.2 Column Equilibration

Equilibrate the column with an appropriate buffer applying approx. 5 column volumes (CV). Ensure the conductivity and pH of the effluent are exactly the same as the equilibration buffer. The equilibration solution can be a neutral pH buffer such as Tris or phosphate buffer, for example 10mM Tris-HCl, 0.5M NaCl, 1mM EDTA (pH 7.4). The affinity interaction between the poly DT on the resin and the poly A of the mRNA molecule is promoted at elevated conductivity, therefore a NaCl concentration of at least 0.2 -0.5M is typically used. The equilibration buffer should present a suitable conductivity to promote mRNA binding.

3.3 Sample preparation and loading

The mRNA sample should be prepared in a buffer solution similar to the equilibration buffer and at the advantageous conductivity. If the RNA sample requires denaturation, heat the sample at 65-70ºC for 10-15min and immediately place it in an ice bath. This treatment helps disrupting secondary structures and may improve purification performance.

mRNA's can differ greatly in size and structure which influence the binding capacity to the Seplife® Oligo dT(20). Sample solubility, concentration, impurity profile as well as the buffer composition affect the binding capacity to Seplife® Oligo dT(20) and should be optimized. Typically the sample application can be performed at a retention time of minimum 3 minutes.

Ensure the sample is filtered (0.22µm or 0.45µm filter) and if possible degassed before application to the column.

After completing the sample application, rinse the column with the equilibration buffer (2-3CV) and with optimized reduced conductivity buffer to improve impurity removal. For example a solution of 10mM Tris-HCl, 0.2M NaCl, 1mM EDTA (pH 7.4) can be applied for 3-5 CV to encourage impurities removal before the sample elution.

3.4 Elution

At reduced conductivity electrostatic repulsion occurs between the polyA and polyT resulting in separation of the pairs. This principle is followed for the mRNA elution from the Seplife® Oligo dT(20) chromatographic resin. Reducing the salt concentration in the buffer or even using water results in the mRNA elution. For example a solution of 10mM Tris-HCl, 1mM EDTA (pH 7.4) 3-5CV or apply 3-5CV water for sample elution. The elution conditions should be optimized to ensure the optimum conditions are

applied for a high purity and recovery of the mRNA.

Elution may be enhanced by heating the elution buffer or the column at 65ºC.

3.5 Regeneration

The Seplife® Oligo dT(20) chromatographic resin should be rinsed with 3-5CV of water or 20% - 30% ethanol solution depending on the severity of the contamination. The regeneration process should be followed by CIP and column wash with the equilibration buffer or equilibrate in storage solution.

3.6 Cleaning-in-place (CIP)

The Seplife® Oligo dT(20) chromatographic resin can be sanitized 3-5CV of 0.1M NaOH solution. If a more severe CIP is require, a 0.5M NaOH solution (3-5CV) can be applied.

After CIP, rinse the column with the equilibration buffer 3-5CVs.

4. Storage

Chromatography resins that are not for immediate use should be stored in 20% ethanol at 2-8 °C. The loaded media should be stored in 20% ethanol at 2-8 °C. Do not freeze.

Allow the bulk media and the packed column to reach room temperature before use.

5. Transportation

Avoid sunlight, rain, and heavy pressure during transportation. It is strictly forbidden to transport with toxic and hazardous materials.

6. Precautions

6.1 Column selection: Theoretically, as long as the column is long enough, the ideal resolution can be obtained, but since the flow rate of the column is related to the pressure gradient, the increase of the column length will slow down the flow rate, broaden the peak, and reduce the resolution. As the diameter increases, the inhomogeneity of liquid flow increases and the resolution decreases significantly.

6.2 Column loading: The loaded column bed must have a flat surface, with no channel flow or air bubbles, otherwise it should be reloaded.

6.3 During the purification process, the pH and conductivity of the elution buffer must be strictly controlled. The sample and chromatography media must be thoroughly equilibrated with equilibration buffer before column chromatography.

6.4 During the elution process, the flow rate should be strictly controlled. It should not be too fast.

6.5 During sample loading and the entire elution process, prevent the column surface from drying out.

7. Ordering information

Product Name	Product Code	Pack Size
Seplife® Oligo dT(20)	PS11225×(50)1-1	25ml
	PS11225×(50)1-2	100ml
	PS11225×(50)1-3	500ml
	PS11225×(50)1-4	1L
	PS11225×(50)1-5	5L
	PS11225×(50)1-6	10L

Production date: See label

Expiry Date: 5 years, under proper storage conditions

Manufacturer: Sunresin New Materials Co. Ltd.

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