KINOVATE LIFE SCIENCES

# Guidelines for Using NittoPhase®HL Solid Support

NittoPhase®HL Solid Support is an optimally designed support for efficient synthesis of pharmaceutical grade oligonucleotides. The cross-linked polystyrene resin has several superior properties needed for obtaining high-quality oligonucleotides in high yield and exceeds the performance of various other supports available in the market. This support has been demonstrated to perform efficiently in both small and large scale synthesis and in a variety of synthesizer apparatus, including OligoProcess™.

## Column Packing Recommendations

### (a) Fixed-bed Columns

Caution: To avoid potential mechanical problems with your synthesizer, pack synthesis column with NittoPhase®HL as a dry powder, using only the recommended quantity per specified volume.

1. Select NittoPhase®HL Solid Support with the desired loading.
2. The amount of NittoPhase®HLSolid Support required for efficient synthesis in a 6.3ml column is set out in the table below. *
3. Using the quantity of support recommended for synthesis in the table below, fill the stainless steel column with dry support, connect the column and start the synthesis program.

**Table 1. NittoPhase®HL solid support packing volume recommendations.***

| RNA Synthesis | Loading (µmol/g) | Packing Volume (6.3ml Column) |
|---|---|---|
| 21-mer | 150 | 0.79 g |
| | 200 | 0.74 g |
| | 250 | 0.69 g |
| **DNA Synthesis** | **Loading (µmol/g)** | **Packing Volume (6.3ml Column)** |
| 20-mer | 300 | 0.83 g |
| | 350 | 0.80 g |
| | 400 | 0.77 g |

* For other size columns, the required packing volume varies in a linear fashion from those listed above. Column packing volume may vary depending on lengths and modifications of oligonucleotides.

### (b) Adjustable Columns

1. Determine the scale of your synthesis.
2. Calculate the amount of NittoPhase®HL Solid Support needed, based on the support loading:

   Amount of support [g] = Scale [µmol] ÷ Loading of the support [µmol/g]
3. Calculate the column volume (CV) using the following formula:

   CV [mL] = Amount of support [g] ÷ 0.109**
4. Calculate the column height h using the formula:

   $h\ [cm] = 4\ CV\ [mL] \div \pi d^2$ d is column diameter in cm
5. Transfer support into column, adjust piston securely at height h.
6. Start synthesis.

### (c) Synthesis Operation

1. Standard synthesis procedures will be applicable with NittoPhase®HL Solid Support.
2. After completion of the synthesis use standard deprotection procedures. Rinse support with ethanol/water (1:1, v/v) for complete recovery.
3. For UnyLinker NittoPhase®HL, 9-11 hours deprotection time in concentrated ammonium hydroxide at 55 °C is sufficient for UnyLinker cleavage.

** Based on 21-mer RNA at 250 µmol/g. Column packing volume may vary depending on lengths and modifications of oligonucleotides.

**For technical quesions, please contact techsupport@kinovate.com**



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