Research

Nucleic Acid Synthesis

Reagents for Phosphoramidite Method

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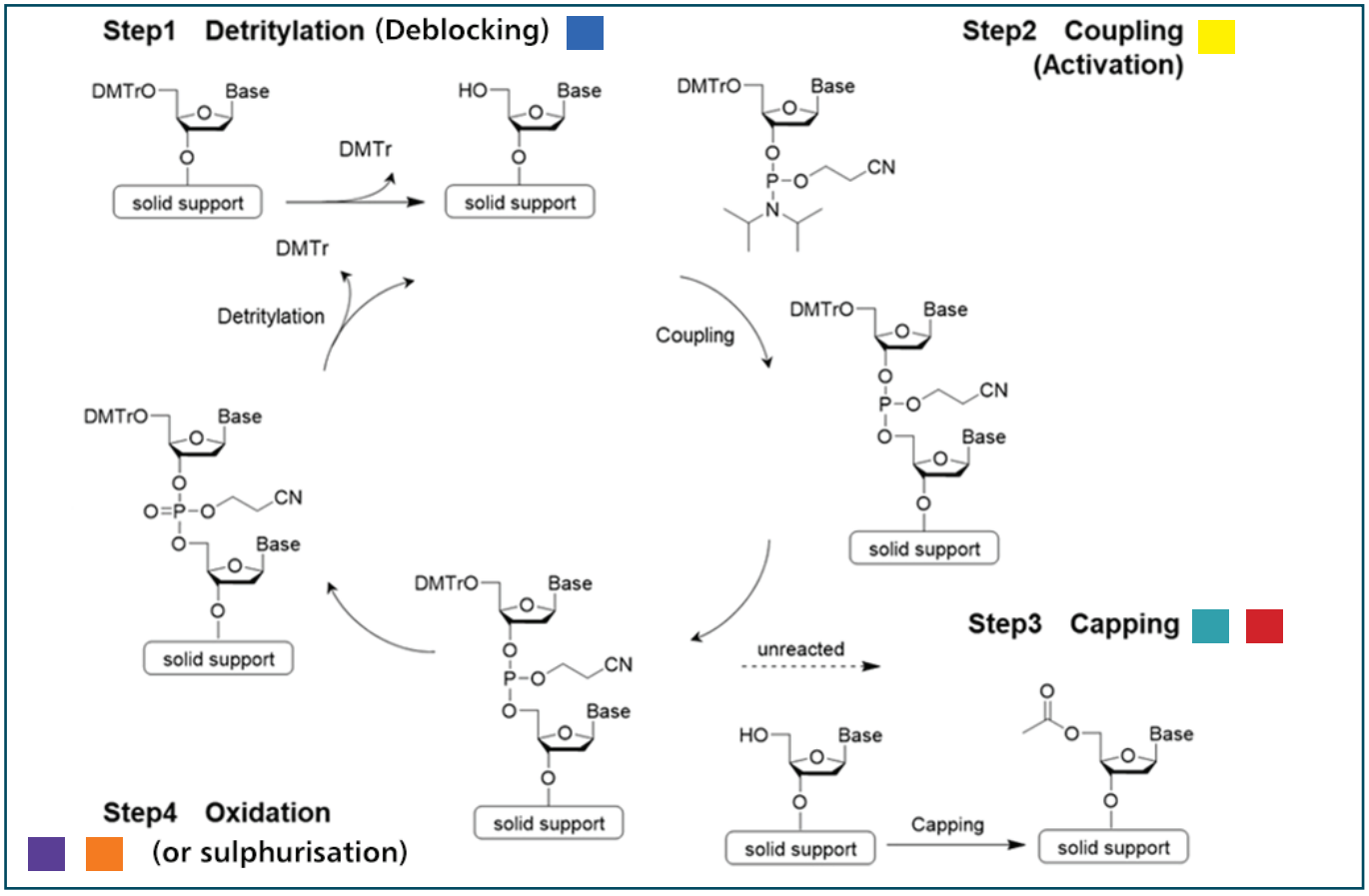
The field of nucleic acid medicine has shown considerable potential for the development of novel disease treatments utilising DNA/RNA. These therapies are particularly promising for cancers and rare genetic disorders, avoiding many of the limitations typical of traditional drugs. Oligonucleotides, the main constituents of these nucleic acids, can be manufactured in-vitro using an automated synthesiser - this typically follows the solid-phase synthesis technique known as the phosphoramidite method.

The fundamental phosphoramidite reaction involves the addition of a phosphoramidite monomer to a solid-phase support and consists of four steps – deblocking/detritylation, amidite activation/coupling, capping, and oxidation/sulphurisation, repeating these steps until the desired strand length is achieved. Synthesised nucleic acids can then be separated and purified by performing solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC).

We offer an extensive range of reagents and consumables on behalf of FUJIFILM Wako Chemicals to supplement this full process from synthesis to purification. These ancillary reagents for the phosphoramidite method are designed for high purity synthesis, support scalability in your workflow, and are conveniently colour-coded for rapid and easy identification in the lab.

- Deblocking reagents
- Activators
- Capping reagents (cap A)
- Capping reagents (cap B)
- Oxidation reagents
- Sulphurising reagents

Phosphoramidite Method – Ancillary Reagents



Deblocking Reagents

As the first step of the phosphoramidite method, detritylation or deblocking involves the removal of the 4,4'-Dimethoxytrityl (DMTr) group from the 5'-OH of the 5'-terminal base at the beginning of each synthesis cycle.

The DMTr group can be easily removed using a weak acid, such as dichloroacetic or trichloroacetic acid, in an inert solution such as toluene or dichloromethane. We offer both traditional deblocking solution formulations to cater to different synthesis requirements.

Product Code	Description	Pack Size
043-34441	Deblocking Solution (3w/v% Dichloroacetic Acid, Toluene Solution)	3L
048-28923	Deblocking Solution-1 (3w/V% Trichloroacetic Acid, Dichloromethane Solution)	1L

Activators

The coupling reaction is the second step of the phosphoramidite method and involves the formation of a phosphite triester linkage between the nucleoside phosphoramidite and the free 5'-OH group of the solid support (first cycle) or the oligonucleotide being synthesised (subsequent cycles).

Before this reaction can occur, the nucleoside phosphoramidite solution must first be activated, typically by an acidic azole catalyst. We offer a broad range of activators for this purpose, available in solution or solid variants.

Product Code	Description	Type	Pack Size
013-19685	Activator Solution-1 (0.25mol/L 4,5-Dicyanoimidazole,Acetonitrile Solution)	Solution	500mL
013-19705	Activator Solution-2 (0.45mol/L 1H-Tetrazole, Acetonitrile Solution)	Solution	500mL
015-20015	Activator Solution-3 (0.25mol/L 5-Benzylthio-1H-tetrazole, Acetonitrile Solution)	Solution	500mL
010-19695	Activator Solution-4 (0.25mol/L 5-Ethylthio-1H-tetrazole, Acetonitrile Solution)	Solution	500mL
044-34851	4,5-Dicyanoimidazole	Solid	5g
051-09491	5-Ethylthio-1H-tetrazole	Solid	5g
130-19221	N-Methylbenzimidazolium Trifluoromethanesulfonate	Solid	5g
161-29041	N-(Phenyl)imidazolium Trifluoromethanesulfonate	Solid	5g

Capping Reagents

After the coupling reaction, the capping step of the synthesis cycle permanently blocks any remaining unreacted 5'-OH groups from further chain elongation, preventing the formation of a shorter, incomplete oligonucleotide.

We provide capping reagents for this step, with strictly controlled moisture content in both the solution and powder forms. In addition to acetyl-based reagents, we also offer a phosphoramidite based reagent designed to reduce the formation of yield-reducing byproducts in the capping reaction.

Product Code	Description	Type	Pack Size
044-35191	N,N-Dicyclohexyl-1,3,2-dioxaphospholan-2-amine	Phosphoramidite-Type	1g
033-25395	Cap A Solution [1-Methylimidazole-Acetonitrile (2:8)]	Acetyl-Type (Acetonitrile Solution)	500mL
034-25401	Cap B Solution [Acetic Anhydride-2,6-Lutidine-Acetonitrile (2:3:5)]	Acetyl-Type (Acetonitrile Solution)	3L
036-25385	Cap B1 Solution [Acetic Anhydride-Acetonitrile (4:6)]	Acetyl-Type (Acetonitrile Solution)	500mL
030-25361	Cap B2 Solution [Pyridine-Acetonitrile (4:6)]	Acetyl-Type (Acetonitrile Solution)	3mL
039-25375	Cap B2 Solution [2,6-Lutidine-Acetonitrile (6:4)]	Acetyl-Type (Acetonitrile Solution)	500mL
036-18991	Cap A Solution-1 (10vol% Acetic anhydride/Tetrahydrofuran Solution)	Acetyl-Type (THF Solution)	3L
030-19011	Cap A Solution-2[Tetrahydrofuran/Acetic Anhydride/Pyridine (8:1:1) Solution]	Acetyl-Type (THF Solution)	3L
033-19001	Cap B Solution-1[Tetrahydrofuran/1-Methylimidazole/Pyridine (8:1:1) Solution]	Acetyl-Type (THF Solution)	3L
037-19021	Cap B Solution-2 (10vol% 1-Methylimidazole/Tetrahydrofuran Solution)	Acetyl-Type (THF Solution)	3L

Oxidation/Sulphurising Reagents

As the phosphite triester linkage formed during the coupling step is slightly unstable, it must be converted into a stable phosphate triester through oxidation. This is achieved using an oxidising agent, typically consisting of iodine and water with a weak base, such as pyridine.

For the synthesis of oligonucleotide phosphorothioates, which are often used in the production of antisense therapies, the oxygen atom in the phosphate moiety is replaced with sulphur. In these instances, the oxidation step is replaced with a sulphurisation step, which is ideally carried out before capping.

We supply reagents for both oxidation and sulphurisation, with a wide range of pack sizes available.

Product Code	Description	Type	Pack Size
150-03515	Oxidising Solution [Iodine Solution (abt. 0.05mol/L)] [Pyridine:Water (9:1)]	Oxidation	500mL
150-03635	Oxidising Solution [Iodine Solution (abt. 0.02mol/L)] [Tetrahydrofuran:Pyridine:Water (78:20:2)]	Oxidation	500mL
156-02451	Oxidation Solution 2	Oxidation	3L
191-18755	Sulphurising Solution (0.05mol/L 5-Phenyl-3H-1,2,4-dithiazol-3-one, Acetonitrile Solution)	Sulphurisation	500mL
194-18745	Sulphurising Solution (0.1mol/L 5-Phenyl-3H-1,2,4-dithiazol-3-one, Acetonitrile Solution)	Sulphurisation	500mL
027-19422	Bis(phenylacetyl) Disulphide	Sulphurisation	25g
012-28582	5-Amino-3H-1,2,4-dithiazole-3-thione	Sulphurisation	25g
166-28251	5-Phenyl-3H-1,2,4-dithiazol-3-one	Sulphurisation	5g

Phosphoramidites

4′-thionucleic acids are chemically modified nucleic acids that exhibit higher duplex-forming ability and greater nuclease resistance compared to their natural counterparts, while maintaining biological equivalence.

These are composed of 4′-thionucleosides, which replace the oxygen atom at the 4′ position of the sugar moiety with a sulphur atom. We offer a range of 4′- thio- compounds designed for the synthesis of 4′-thionucleic acids.

Product Code	Description	Pack Size
042-34891	DMT-2′-O-TBDMS-4′-thio-rA(Bz) Phosphoramidite (mixture of isomers)	250mg
045-34901	DMT-2′-O-TBDMS-4′-thio-rG(iBu) Phosphoramidite (mixture of isomers)	250mg
042-34911	DMT-2′-O-TBDMS-4′-thio-rC(Bz) Phosphoramidite (mixture of isomers)	250mg
049-34921	DMT-2′-O-TBDMS-4′-thio-rU Phosphoramidite (mixture of isomers)	250mg

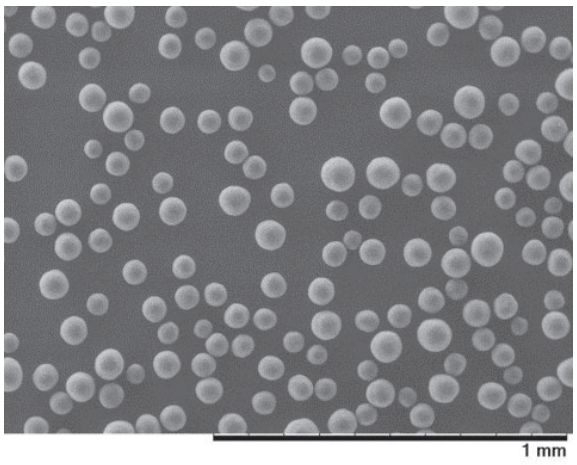
Solid Supports

Throughout the entire assembly process, the oligonucleotide is continuously attached to a solid support. We offer the Aminolinker range of highly cross-linked polystyrene supports, which deliver high coupling and reagent washing efficiency throughout the process and produce oligonucleotides of higher purity compared to other supports.

These supports feature a porous, low-swelling polystyrene matrix, minimising volume change, and are of superior physical strength to reduce damage during synthesis. The support is provided in its native form and functionalised with an amino linker – nucleosides and universal linkers can then be freely conjugated to the support for oligonucleotide synthesis.

In addition to the Aminolinker support, we also supply the PT Linker range of phenanthrene-type universal linkers. Universal linkers enable a single, underivatised solid support to be used irrespective of the sequence of the oligonucleotide to be synthesised.

The PT linker consists of a bicyclic 1,2-diol structure containing a phenanthrene moiety and a succinyl unit – this connects the solid support to the phenanthrene via an amide bond. This linker demonstrates high lipophilicity and UV-detectability, facilitating easy separation of the targeted oligonucleotide from linker-derived impurities.



Product Code	Description	Pack Size
018-28981	Aminolinker PS 1000A, Standard Loading	1mg
015-29111	Aminolinker PS 2000A, Standard Loading	1mg
012-29121	Aminolinker PS 3000A, Standard Loading	1mg
169-29841	PT Linker PS 1000A, Standard Loading	1mg
160-30001	PT Linker CPG 2000A, Standard Loading	250mg

Chemical Phosphorylation Reagent

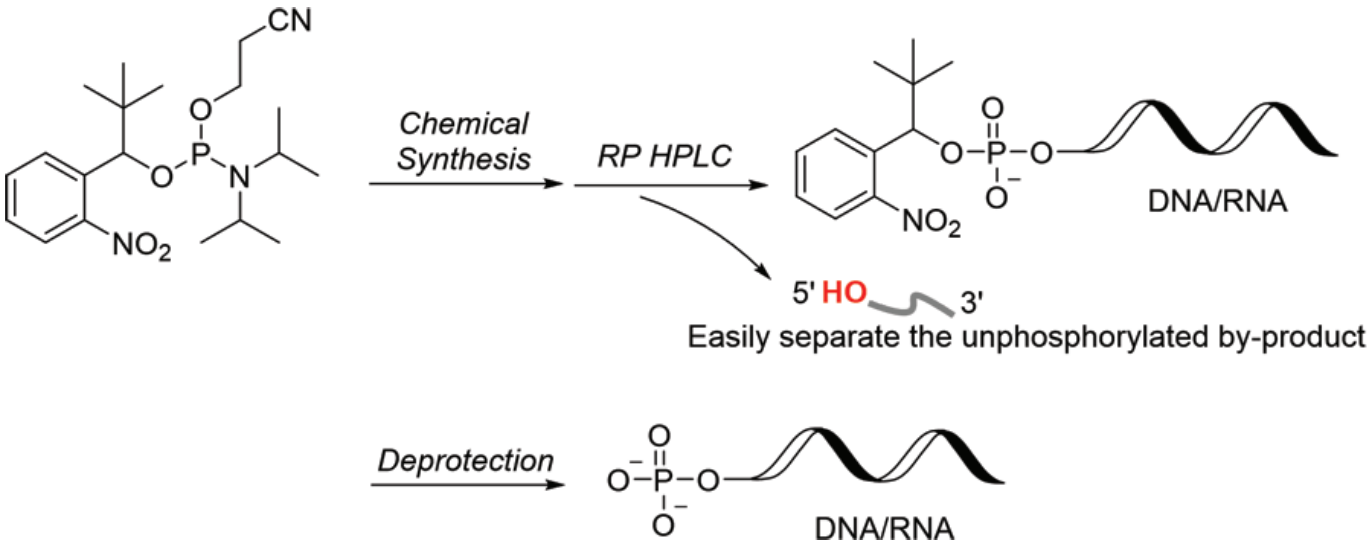
5’-monophosphorylated oligonucleotides, which play a key role in nucleic acid functionalisation and ligation reactions, may be synthesised by introducing a monophosphate group at the 5’-end of oligonucleotides. This has been typically achieved through enzymatic phosphorylation; however, this methodology can suffer from variable efficiency due to high substrate specificity.

Chemical phosphorylation has therefore presented itself as an effective alternative to conventional enzymatic phosphorylation - by directly introducing the monophosphate group to the 5’-end, this avoids the downsides of enzyme usage and enables highly efficient synthesis of 5’-monophosphorylated RNA.

We offer an innovative chemical phosphorylation reagent designed for the high-purity synthesis of 5’-monophosphorylated oligonucleotides using the phosphoramidite method, with a coupling efficiency of up to 99%.

The reagent introduces a hydrophobic nitrobenzyl tag, enabling efficient separation and purification of the target oligonucleotide by reverse phase HPLC. This uses the same principle as DMT-ON purification, resulting in markedly improved purity. The UV-induced deprotection step suppresses degradation of RNA, making it ideal for 5’-monophosphorylated RNA synthesis.

This reagent demonstrates key benefits for use in the development of novel mRNA therapeutics, such as cancer vaccines and genetic disease treatments.



Product Code	Description	Pack Size
035-26411	2-Cyanoethyl [2,2-dimethyl-1-(2-nitrophenyl)propyl] N,N-Diisopropylphosphoramidite	250mg

Solid-Phase Extraction Columns

Solid-phase extraction (SPE) is a widely used technique for straightforward purification during oligonucleotide synthesis. SPE is ideal for high-throughput processing and is designed for use when the DMTr group is retained at the end of synthesis, and as a pre-treatment step for HPLC.

Presep® DNA/RNA SPE columns are dedicated for the separation and purification of nucleic acids. These columns demonstrate:

- Sample load 3-5 times higher than commercially available pretreatment columns
- Superior deprotection efficiency
- Excellent recovery rate
- High purity

Product Code	Description	Pack Size
290-36691	PresepR DNA/RNA Type A (85mg/1mL) (1)	20pcs
290-36711	PresepR DNA/RNA Type A (255mg/3mL) (2)	20pcs
292-36891	PresepR DNA/RNA Type A (1,000mg/15mL) (3)	10pcs
292-36911	PresepR DNA/RNA Type A (1,700mg/25mL) (4)	10pcs
299-36921	PresepR DNA/RNA Type A (5,100mg/70mL) (5)	10pcs

HPLC Columns for Nucleic Acid Purification

High-performance liquid chromatography (HPLC) is widely used for oligonucleotide purification and analysis. Wakopak® Wakosil®-DNA is a dedicated column developed specifically for the analysis and fractionation of oligonucleotides, performing efficient analysis and fractionation of synthetic single-stranded DNA up to several tens of mer for each 1 mer difference.

Wakosil-DNA columns exhibit high separation capacity and recovery rates to ensure the highest quality purification. Supplied with DuPont style joints, with Water's style joints also available for 7.5mm and 10mm models.

Product Code	Description	Pack Size
239-59321	Wakopak Wakosil-DNA 4.6*150mm (D)	Each
236-59331	Wakopak Wakosil-DNA 4.6*250mm (D)	Each
237-61711	Wakopak Wakosil-DNA 7.5*250mm (D)	Each
234-61721	Wakopak Wakosil-DNA 10*250mm (D)	Each

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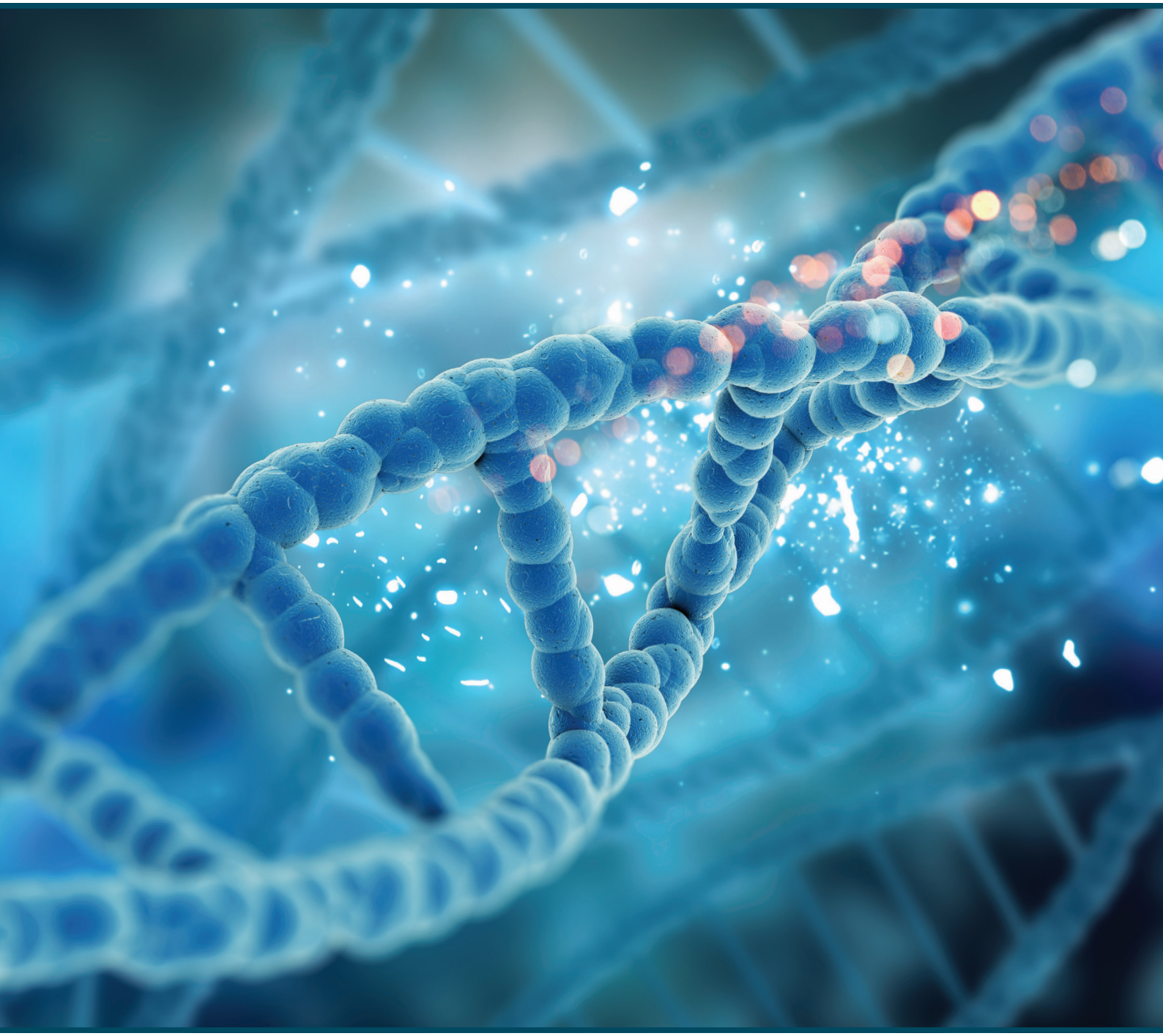

Visit www.alphalabs.co.uk/nucleic-acid-synthesis for more information.

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JB466A / 07.26