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Product Information

High Throughput Crystallization Kit for Nucleic Acids
Product Number 02828
Store at 4°C

TECHNICAL BULLETIN

Application

The Crystallization Kit for RNA is an empirical screening method for the direct determination of suitable crystallization conditions for nucleic acids.

The Kit is based on a combination of conditions from our crystallization kit for DNA and our crystallization kit for RNA.

One part presents a screen according to the described method of Nowakowski, et al.(1) The solution and crystallization conditions are empirically derived based on known or published crystallization conditions of nucleic acids, especially of DNA enzymes or other complex nucleic acids. (1-5)
The other part covers conditions described by Baeyens, Jancarik and Holbrook. (6) These ready-made solutions represent a screen of conditions, from which many were observed to produce crystals from DNA oligomers and transfer RNA. Combined in one screening kit 96 conditions provide an efficient tool for evaluation of suitable conditions for various kinds of nucleic acids.

This kit comes in a deepwell block, to allow convenient handling by either automated liquid handling or multi channel pipets.

Quality of reagents – a key to success

All the reagents within this kit are formulated using high purity reagents. These reagents are specially purified and analyzed to ensure the absence of any significant traces of ions or other impurities. This enables the reliable and precise formulation of crystallization conditions as required for best results. There are many experiences where the Biochemika ultra chemicals (former Microselect grade) have successfully been used for different crystallization methods. All solutions are sterile filtered using 0.22 micron filters.
All solutions are available separately as 100 ml bottles. Larger quantities are available on request.

Storage/Stability

It is recommended that the reagents of this kit be stored at 4 °C. Storage at –20 °C will not adversely affect the kit reagents, but storage at –20 °C may cause precipitation esp. of some concentrated phosphate solutions, requiring re-solubilization by shaking, higher temperature or sonic waves. The reagents as supplied are stable at room temperature for short-term storage. Kit reagents should not be set under ultraviolet light to protect them from microorganisms.

Sample Preparation Instruction

The sample has to be as pure as possible and free of amorphous material or other particles. The purity should be >95%. Amorphous material can be removed by centrifugation or micro-filtration. (8-10) For the stock solution it is recommended to have a high concentration, at least 5 mg/ml. It should be an aqueous solution without or with very low buffer capacity (e.g. below 10 mM buffer substance), as otherwise buffer would influence the pH of crystallization experiments. This solution may contain a low concentration of EDTA (e.g. 0.1 mM). RNA molecules are much more subject to hydrolysis than DNA molecules. RNAses are more frequently a problem than DNAses. Sterile techniques should be used for the whole sample preparation (7).

Nucleic acids may be stabilized by addition of spermine or spermidine (typically around 1 mM) or other stabilizing agents (e.g. Ectoin or Hydroxyectoin). Typically spermine or spermi-dine are used and added to the reservoir.

DNA samples should be heated to 60-70°C for 10 min, cooled down to room temperature again, centrifuged and microfiltrated before use.

Handling and Procedures

Before use, centrifugation of the deepwell block with a swing-out rotor centrifuge is suggested, to remove solutions from the cover foil. Centrifugation prevents cross contamination while opening the plate. Otherwise drops under the cover foil may be shaken down. The film should be very gently peeled from the plate.

First open the strap and turn it down to the surface of the deep well plate (Fig. 1). Then smoothly pull the strap in horizontal direction so that the aluminium foil moves parallel to the plate surface (Fig. 2) to avoid contamination of the wells by drops hanging on the foil.

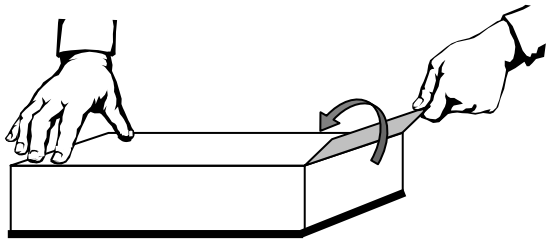

Fig. 1

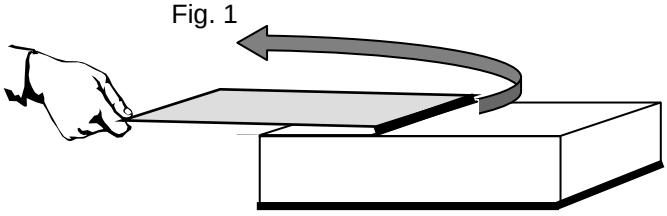

Fig. 2

Instead of peeling the film, it can also be left intact and pierced to take out reagent.

The following procedure describes the use of this kit with the Sitting Drop Vapor Diffusion method.

For manual handling, either a 8 or 12 channel pipet can be used to pipet the recommended volume (50-100 µl) into the reservoirs of a 96 well crystallization plate. Pipet tips should be changed for each set of conditions. But several crystallization plates may be prepared at once (row or column wise) to save tips.

Transfer 50 nl to 2 µl of crystallization reagent from reservoir to the sitting drop well (for each well). Again a 8 or 12 channel pipet may be used. Pipet tips should be changed for each well or set of wells.

Pipet same volume of sample (50 nl to 2 µl) into each sitting drop well. Mixing can be performed by gently aspirating and dispensing the drop several times.

Seal the crystallization plate as you usually do or according to the suppliers recommendations. Seal the deepwell block using foils or cover mats (for short term storage). You may use the enclosed aluminium foils. Simply pull the aluminium foil from the protective film and put the foil on the plate by pressing it onto the surface with evenly spread pressure. Take care that the foil is flush with one side of the plate and overhanging on the other side. To simplify the reopening of the kit put a piece of adhesive tape on the underside of the overhanging aluminium foil.

Please note: When kits are sealed manually we highly recommend to store the kits in upright position at 4°C in the dark.

Sample solubility is also temperature dependent. Allthough most crystallizations have been achieved at room temperature, in many cases different temperatures have lead to success. Comparison of results of screening at two different temperatures (4 °C and room tem-perature) helps determine the magnitude of temperature effects on sample solubility. Temperature may be an important parameter in the optimization procedure.
Due to the standard format of deepwell blocks, they are suitable for any type of standard liquid handler (working with single tip or needle up to 96 parallel channels)

Observation

Drops are typically observed by a stereo microscope at 10 to 100x magnification. Record all observations by scanning every droplet on the slides.

Scan the focal plane for small crystals and record observations for all droplets. Scan the first time shortly after the screen is set up. Then for the first 5-10 days, information may be recorded daily and, thereafter, on a weekly basis. Records should include the clarity of the droplet (clear, precipitate, or crystals), along descriptive phrases and a numerical scale. The following are possible examples (see also observation sheet):
10(= crystal grown 1 D) shower of needles, yellow
6(= gelatinous protein precipitate) red/brown
1(= drop is clear), green
7 (= fully precipitated dark color) dark green
It can be useful to write down the largest crystal size!

Results and Interpretation

A clear drop may be an indication that the drop has not yet reached its final state. If the drop remains clear after 2 to 4 weeks, the relative sample and reagent supersaturation may be too low. If a majority of drops remain clear, consider repeating the entire screen using a sample at higher concentration.

There are several reasons for precipitation in a drop. A precipitate can indicate that the sample or precipitant concentration is too high (precipi-tation within 1 day) or it is not the preferred crystallization condition (within a few days). In the case of too high concentration, repeat the screen with lower protein concentration. If a majority of drops contain a precipitate with no crystals present, consider diluting the sample and repeating the entire screen. In the case of precipitation problems for several screens, it may be useful to dilute the precipitant in the reagent.
Precipitation may also be an indication that the target molecule has denatured. It may be necessary to take steps to stabilize the nucleic acids. These could include the addition of reducing agents, glycerol or salts.
Sample purity may also cause precipitation. Low sample purity, aggregation, or a heterogeneous preparation may be responsible for precipitation. In these cases, further sample purification is required.
It is possible that a crystal may form out of a precipitate. Crystals can grow extremely fast, in few minutes, or may require much more time, up to a few months. This is the reason that crystallization plates should never be trashed, or a drop disregarded too early. Store and record the plates until the drops are dried out.

It is recommended that a high power microscope be used to examine the precipitate between crossed polarizing lenses. True amorphous precipitates do not glow. Birefringent micro-crystalline precipitates can glow as a result of the plane of light polarization. It may be possible to use streak seeding to produce larger crystals from microcrystalline precipitates.

Screens, which produce crystals, provide the first clues regarding conditions for crystallization. It may be necessary to optimize these conditions to produce crystals with the proper size and quantity for analysis. The following parameters should be considered during optimization: pH, salt type and concentration, precipitant type and concentration, temperature, sample concentration, and other additives.

References

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5. Scott, W.G., et al, J. Mol. Biol. 250, 327-332, 1995.
6. Use of Low-Molecular-Weight Polyethylene Glycol in the Crystallization of RNA Oligomers, Baeyens, K. J., Jancarik, J., Holbrook, Acty Cryst., D50, 764-767, 1994.
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10. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990

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A1	28674	50 mM HEPES Ph 7.5, 80 mM $MgCl_2$, 2.5 mM Spermine
A2	51189	50 mM Cacodylate pH 7.0, 20 mM $MgCl_2$, 1.0 mM Spermine, 1.0 mM Cobalt(III)hexamine, 15% Ethanol
A3	89269	50 mM Cacodylate pH 7.0, 9 mM $MgCl_2$, 2.25 mM Spermine, 1.8 mM Cobalt(III)hexamine, 0.9 mM Spermidine, 5% PEG 400
A4	19485	50 mM Cacodylate pH 6.5, 30 mM $MgCl_2$, 1.0 mM Spermine, 1.3 M Lithium sulfate
A5	66017	50 mM HEPES pH 7.5, 15 mM $MgCl_2$, 1.0 mM Spermine, 1.0 M Ammonium sulfate
A6	75771	50 mM TRIS pH 8.0, 200 mM $MgCl_2$, 15 % Ethanol
A7	78989	50 mM Cacodylate pH 6.5, 20 mM NaCl, 5 mM $MgCl_2$, 2 mM Spermine hydrochloride, , 35% 2-Methyl-2,4-pentandiol
A8	97067	20 mM NaCl, 5 mM $MgCl_2$, 15% Isopropanol
A9	73078	50 mM Cacodylate pH 6.5, 5 mM $MgCl_2$, 5 mM Spermine hydrochloride, 35 % PEG 4000
A10	68115	50 mM Cacodylate pH 6.5, 20 mM Lithium chloride, 5 mM $MgCl_2$, 35 % 2-Methyl-2,4-pentandiol
A11	06391	50 mM TRIS pH 8.0, 20 mM Ammonium chloride, 1 mM Samarium(III)-chlorid, 20 % Ethanol
A12	42652	50 mM Cacodylate pH 6.0, 1 mM Bariu mchloride, 1 mM Samarium(III)-chloride, 35 % PEG 4000
B1	76475	0.05 M Cacodylate pH 6.0, 18 mM $MgCl_2$, 2.25 mM Spermine, 1 mM $CuSO_4$, 9% Isopropanol
B2	19693	50 mM Cacodylate pH 7.0, 5 mM $MgCl_2$, 1.0 mM Spermidine, 10% tert-Butanol
B3	56961	50 mM Cacodylate pH 6.5, 10 mM $MgCl_2$, 2.5 mM Spermine, 1 mM Copper sulfate, 10% Isopropanol
B4	68446	50 mM Cacodylate pH 6.0, 200 mM Calciumacetat, 5% Isopropanol
B5	18154	50 mM Cacodylate pH 6.0, 200 mM Calcium acetate, 2.5 M NaCl
B6	92213	50 mM Cacodylate pH 6.0, 15 mM $MgCl_2$, 5.0 Spermidine, 2.0 M Lithium sulfate
B7	89369	50 mM HEPES pH 7.0, 5 mM $MgCl_2$, 35% 2-Methyl-2,4-pentandiol
B8	97069	50 mM TRIS pH 7.5, 5 mM Cadmium chloride, 2 mM Spermin hydrochloride, 15% Isopropanol

B9	79993	50 mM TRIS pH 7.5, 20 mM NaCl, 35 % PEG 8000
B10	92102	50mM Cacodylate pH 6.0, 5 mM Manganese chloride, 1 mM Samarium(III)-chlorid, 5 mM Argininamide, 30 % 2-Methyl-2,4-pentandiol
B11	05639	50 mM Cacodylate pH 6.0, 20 mM Argininamid, 1 mM Bariumchlorid, 2 mM Spermidine hydrochloride 30 % PEG 400
B12	22664	50 mM TRIS pH 8.0, 20 mM NaCl, 1 mM Ruthenium(III)-chloride, 35 % PEG 8000
C1	42393	50 mM Cacodylate pH 6.5, 18 mM $MgCl_2$, 0.9 mM Spermine, 1.8 mM Cobalt(III)hexamine, 9% Isopropanol
C2	93084	50 mM Cacodylate pH 7.0, 30 mM $MgCl_2$, 2.5 mM Spermine, 5% PEG 400
C3	91373	50 mM Cacodylate pH 6.0, 20 mM $MgCl_2$, 1.0 mM Spermine, 2 mM $CaCl_2$, 10% 1,5-Hexandiol
C4	80215	50 mM Cacodylate pH 6.5, 100 mM $MgCl_2$, 1.0 mM Kobalt(III)hexamin, 10% Ethanol
C5	42794	50 mM Cacodylate pH 6.0, 200 mM Calcium acetate, 1.0 mM Cobalt(III)hexamine, 2.0 M LiCl
C6	53245	50 mM Cacodylate pH 6.0, 20 mM Magnesium acetate, 0.5 mM Spermine, 100 mM NaCl, 25% MPD
C7	91854	50 mM TRIS pH 7.5, 20 mM KCl, 2 mM Spermine hydrochloride, 30% 2-Methyl-2,4-pentandiol
C8	69408	50 mM HEPES pH 7.0, 20 mM Ammonium chloride, 30 % PEG 400
C9	79182	50 mM HEPES pH 7.0, 20 mM KCl, 5 mM $MgCl_2$, 1.2 M Ammonium sulfate
C10	16953	50 mM TRIS pH 8.0, 20 mM NaCl, 5 mM Zinc chloride, 30 % 2-Methyl-2,4-pentandiol
C11	05673	50 mM Cacodylate pH 6.5, 20 mM KCl, 5 mM Calcium chloride, 30 % PEG 400
C12	40337	50 mM HEPES pH 7.0, 5 mM Zinc chloride, 35 % PEG 8000
D1	89253	50 mM Cacodylate pH 6.5, 18 mM $MgCl_2$, 2.25 mM Spermine, 9% Isopropanol
D2	30517	50 mM Cacodylate pH 6.5, 100 mM $MgCl_2$, 2.0 mM Cobalt(III)-hexamine, 5% Isopropanol
D3	30193	50 mM HEPES pH 7.5, 15 mM $MgCl_2$, 1.0 mM Spermidine, 10 % Dioxan
D4	19348	50 mM Cacodylate pH 6.0, 10 mM $MgCl_2$, 2.5 mM Spermidine, 2.5 M NaCl
D5	40377	50 mM Cacodylate pH 6.5, 15 mM $MgCl_2$, 5.0 mM Spermidine, 1.0 Cobalt(III)hexamin, 2.0 M NaCl
D6	43374	50 mM Sodium succinat pH 5.5, 20 mM $MgCl_2$, 0.5 mM Spermine, 3.0 Ammonium sulfate
D7	78481	50 mM HEPES pH 7.0, 20 mM KCl, 5 mM Manganese chloride, 35% 2-Methyl-2,4-pentandiol
D8	74094	50 mM Cacodylate pH 6.5, 20 mM NaCl, 5 mM $MgCl_2$, 2 mM Spermine hydrochloride, 35 % PEG 400
D9	69566	50 mM Cacodylate pH 6.5, 20 mM NaCl, 1.2 M Ammonium sulfate
D10	39593	50 mM TRIS pH 7.5, 1 mM Ruthenium(III)-chloride, 30 % 2-Methyl-2,4-pentandiol
D11	02969	50 mM HEPES pH 7.0, 1 mM Cobalthexamine chloride, 35 % PEG 400
D12	57924	50 mM Cacodylate pH 6.5, 5 mM Calcium chloride, 2 mM Spermidine hydrochloride, 20 % Dioxane
E1	67516	50 mM Cacodylate pH 7.0, 18 mM $MgCl_2$, 2.25 mM Spermine, 0.9 mM Cobalt(III)hexamine, 4.5% MPD
E2	51804	50 mM TRIS pH 8.0, 10 mM $MgCl_2$, 1.0 mM Cobalt(III)hexamine, 20% Ethanol
E3	67198	50 mM Cacodylate pH 6.0, 15 mM $MgCl_2$, 3.0 mM Spermine, 10% PEG 400
E4	16829	50 mM Cacodylate pH 6.5, 10 mM $MgCl_2$, 200 mM Sodium citrat, 5% Isopropanol
E5	15873	50 mM Cacodylate pH 6.5, 200 mM $MgCl_2$, 100 mM NaCl, 20% PEG 1000
E6	44401	50 mM Cacodylate pH 6.5, 5 mM Cobalt(III)hexamine, 2.5 M KCl
E7	52239	50 mM Cacodylate pH 6.5, 1 mM Barium chloride, 20 mM Ammonium chloride, 30% MPD
E8	44789	50 mM TRIS pH 7.5, 20 mM NaCl, 5 mM $MgCl_2$, 35 % PEG 400
E9	52266	50 mM Cacodylate pH 6.5, 20 mM KCl, 5 mM $MgCl_2$, 1.2 M Sodium acetate
E10	53884	50 mM HEPES pH 7.0, 1 mM Cobalthexamine chloride 30 %, 5 mM Argininamide, 2-Methyl-2,4-pentandiol, 10 % PEG 1000
E11	03268	50 mM TRIS pH 8.0, 20 mM NaCl, 1 mM Ruthenium(III)-chloride, 35 % PEG 400
E12	50241	50 mM Cacodylate pH 6.0, 20 mM Lithium chloride, 5 mM Cobalt(II)-chloride, 20 % Dioxane
F1	90746	50 mM Cacodylate pH 6.5, 36 mM $MgCl_2$, 2.25 mM Spermine, 5% PEG 400
F2	90748	50 mM HEPES pH 7.5, 20 mM $MgCl_2$, 1.0 mM Spermine, 5 % PEG 8000
F3	97863	50 mM Cacodylate pH 6.5, 2.5 mM Spermine, 18 mM $CaCl_2$, 9% 2-Propanol
F4	44046	50 mM Cacodylate pH 6.5, 15 mM $MgCl_2$, 10.0 mM Spermine, 2.0 M Lithiumsulfat
F5	42029	50 mM TRIS pH 7.5, 50 mM $MgCl_2$, 1.0 M Sodium tartrate
F6	05556	50 mM Cacodylate pH 6.5, 50 mM $MgCl_2$, 2.0 mM Cobalt(III)hexamine, 1.5 M Lithium sulfate
F7	73384	50 mM Cacodylate pH 6.5, 20 mM NaCl, 1 mM Cadmium chloride, 35% 2-Methyl-2,4-pentandiol
F8	5336	50 mM Cacodylate pH 6.5, 20 mM KCl, 1 mM Cadmium chloride, 35 % PEG 400
F9	41341	50 mM HEPES pH 7.0, 1 mM Barium chloride, 1.2 M Sodium acetate
F10	05638	50 mM Cacodylate pH 6.0, 20 mM Ammonium chloride, 2 mM Putrescine hydrochloride, 30 % 2-

		Methyl-2,4-pentandiol, 10 % PEG 400
F11	06531	50 mM Cacodylate pH 6.5, 20 mM Lithium chloride, 5 mM Cobalt(II)-chloride, 35 % PEG 600
F12	07781	50 mM TRIS pH 7.5, 20 mM Ammoniumchlorid, 20 % Dioxane
G1	89264	50 mM Sodium succiat pH 5.5, 10 mM $MgCl_2$, 2.0 mM Cobalt(III)Hexamin, 10% Isopropanol
G2	95109	50 mM Cacodylate pH 6.0, 20 mM $MgCl_2$, 2.5 mM Spermine, 5% PEG 4000
G3	55784	50 mM Cacodylate pH 6.5, 2.0 mM Spermine, 1.0 mM Cobalt(III)hexamine, 80 mM $CaCl_2$
G4	73519	50 mM Cacodylate pH 6.5, 20 mM $MgCl_2$, 1.0 mM Spermine, 2.0 M Ammonium sulfate
G5	44259	50 mM TRIS pH 7.5, 200 mM $MgCl_2$, 2.5 M NaCl
G6	04359	50 mM Cacodylate pH 6.5, 1.0 mM Spermine, 2.0 mM Cobalt(III)hexamine, 30 mM $CaCl_2$, 2.0 M LiCl
G7	97066	50 mM HEPES pH 7.0, 20 mM NaCl, 5 mM Zinc chloride, 30% 2-Methyl-2,4-pentandiol
G8	73942	50 mM HEPES pH 7.0, 20 mM Ammonium chloride, 5 mM $MgCl_2$, 35 % PEG 600
G9	67627	50 mM HEPES pH 7.0, 20 mM Sodium chloride, 40 % tert-Butanol
G10	05092	50 mM TRIS pH 8.0, 20 mM KCl, 5 mM $MgCl_2$, 30 % 2-Methyl-2,4-pentandiol, 5 % Isopropanol
G11	11076	1 mM Cobalthexamine chloride, 2 mM Putrescin hydrochloride, 35 % PEG 600
G12	36419	50 mM HEPES pH 7.0, 1 mM Cadmiumchlorid, 2 mM Spermine hydrochloride, 40 % tert-Butanol
H1	90971	50 mM Cacodylate pH 6.0, 20 mM $MgCl_2$, 1.0 mM Spermine, 15% Ethanol
H2	89263	50 mM Cacodylate pH 6.0, 10 mM $MgCl_2$, 2.5 mM Spermine, 5 mM CaCl, 10% Isopropanol
H3	07312	50 mM Cacodylate pH 6.5, 5 mM $MgCl_2$ 2.5 mM Cobalt(III)hexamine
H4	79853	50 mM Cacodylate pH 6.5, 10 mM $MgCl_2$, 1.5 mM Spermine, 3.0 Ammonium sulfate
H5	41172	50 mM Cacodylate pH 6.0, 200 mM $MgCl_2$, 2.5 M KCl
H6	66953	50 mM Cacodylate pH 6.5, 10 mM $MgCl_2$, 50 mM Spermine
H7	97068	50 mM Cacodylate pH 6.5, 15% Isopropanol
H8	68121	50 mM Cacodylate pH 6.5, 20 mM NaCl, 5 mM $MgCl_2$, 30 % PEG 1000
H9	92448	5 mM $MgCl_2$, 2 mM Spermine hydrochloride, 15 % Acetone
H10	05630	50 mM Cacodylate pH 6.0, 1 mM Barium chloride, 20 % Ethanol
H11	40879	50 mM Cacodylate pH 6.0, 20 mM Argininamide, 1 mM Cadmium chloride, 30 % PEG 1000
H12	41718	50 mM TRIS pH 7.5, 20 mM KCl, 1 mM Barium chloride, 15 % Acetone

02828 High Throughput Crystallization Kit for Nucleic Acids – Observation Sheet

Sample description: _____ Date: _____
concentration: _____ Incubation Temperature: _____
buffer: _____ Reservoir Volume: _____

Drop contains : Crystallization Reagent_____ul Sample_____ul Additive (name)_____(volume)_____ul

	precipitate without irefringent and edges	**precipitates shows birefringent or has edges**
1 drop is clear	3 mostly clear drop	7 sperulites or small structures maybe edges
2 drop contains non-protein particles	4 fully precipitated dark colour	8 crystal grown 1 D
	5 gelatinous protein precipitate	9 crystal grown 2 D
	6 phase separation	10 crystal grown 3 D

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												