Crystallization Kit for RNA
Product Number **18839**
Store at 2-8 °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 developed according to the described method of Baeyens, Jancarik and Holbrook (1) These ready-made solutions represent a screen of conditions, from which many were observed to produce crystals from DNA oligomers and transfer RNA. This screen provides high efficiency in crystallization of RNA oligomers. Thus it provides a completion of our crystallization kit for DNA.

Composition

The Crystallization Kit for RNA consists of 48 different formulations covering a range of different buffers (pH 6.0-8.0), different types of precipitants (esp. PEG 400 and MPD), and inorganic salts including magnesium salts. All solutions are sterile filtered using 0.22 micron filters. The kit contains 10 ml of each formulation, but all solutions are available separately as 100 ml bottles. Larger quantities are available on request.

Quality of reagents – the key to success

Crystallization Kit for nucleic acids reagents are formulated using high purity reagents (mostly Biochemika ultra/MicroSelect from Fluka). 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 instances where the Biochemika ultra/MicroSelect chemicals have successfully been used for different crystallization methods.

Precautions and Disclaimer

This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

It is recommended that the reagents of this kit be stored at 2-8 °C. Storage at –20 °C will not adversely affect the kit reagents and 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. (2-4) 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), or even without buffer, 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 (5).

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 Spermidine are used and added to the reservoir.

The sample should be heated to 60-70°C for 10 min, cooled down to room temperature again, centrifuged and microfiltrated.

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SIGMA

Procedures

The application method described below is the most well-known method of crystallization: the Hanging Drop Vapor Diffusion method. Other methods like the Sitting Drop, Microdialysis, MicroBatch, and Sandwich Drop methods are also applicable for the Crystallization Kit for nucleic acids. The sitting drop method is being used more and more. It will probably gain further importance since the hanging drop procedure is difficult to use with automated procedures. Directions for the Hanging, Sitting Drop and other crystallization methods are available from Fluka Technical Service.

1. Take two 24-Well plates (from Stratech, ICN Biomedicals, Costar, Falcon, Molecular Dimensions Ltd.). It is recommended that on each upper edge of the 24 wells should be put a thin film of grease. This ensures that the cover slide doesn't shift. Prepare fifty wells for one full screen of a protein. See figure 1 below.

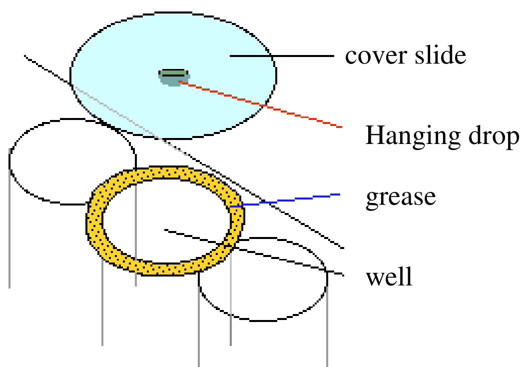

figure 1: well plate

2. Pipet 800 µl of each reagent into the fifty wells. *Use always a clean pipet tip for each reagent!*

3. 2 µl of sample are put into the middle of a clean, siliconized 22 mm (18 mm if you use Costar plate) diameter cover slide (Molecular Dimensions Ltd, Stratech). See figure 1. It is also possible to take a square cover slide.

4. Pipet 2 µl of the corresponding crystallization reagent from each well into the sample droplet. Mix with caution by dispensing and aspirating the droplet with the pipet. Avoid foaming by keeping the tip in the drop.

5. Invert the cover slide and droplet directly over the well containing the appropriate reagent and place the cover slide on top of the well. Make sure the grease seal is complete between the cover slide and the edge of the well.

6. Repeat steps 3. to 5. for all reagents.

7. A recommended practice is to perform the crystallization screen at 4°C and at room temperature if there is enough sample. Incubate and store the plates in a place with stable temperature and free of vibration.

Sample solubility is also temperature dependent. Although most crystallizations have been achieved at room temperature, in many cases different temperatures have led to success. Comparison of results of screening at two different temperatures (4 °C and room temperature) helps determine the magnitude of temperature effects on sample solubility. Temperature may be an important parameter in the optimization procedure.

Observation

Drops are typically observed by a stereo microscope at 10 to 100X. 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 with 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 precipitate) red/brown
1(= drop is clear), green
7 (= fully precipitated dark color) dark green
It is also useful to write down the largest crystal size!

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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 (precipitation 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 microcrystalline 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

1. 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.
2. Crystallization of nucleic acids and proteins, A. Ducruix and R. Giege eds., The Practical Approach Series, Oxford Univ. Press, 1992.
3. Current approaches to macromolecular crystallization. McPherson, A. Eur. J. Biochem. 189, 1.23, 1990.
4. Protein and Nucleic Acid Crystallization Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.
5. Preparation and Crystallization of RNA: A Sparse Matrix Approach, Kundrot, C., in Methods of Enzymology, 276, 143-156, 1997.

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18839 Crystallization Kit for RNA Observation Sheet

Sample description: ________________________________ Date: ______________________________
concentration: ______________________________ Incubation Temperature: ______________
buffer: ____________________________________ Reservoir Volume: ___________________
Drop contains : Crystallization Reagent_______ul Sample_______ul Additive (name)______________(volume)_____ul

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

No.	**Fluka No.**	**Reagent composition**	**Date:**	**Date:**	**Date:**	**Date:**	**Date:**
K1	**78989**	50 mM Cacodylate pH 6.5, 20 mM NaCl, 5 mM $MgCl_2$, 2 mM Spermine hydrochloride, , 35% 2-Methyl-2,4-pentandiol					
K2	**89369**	50 mM HEPES pH 7.0, 5 mM $MgCl_2$, 35% 2-Methyl-2,4-pentandiol					
K3	**91854**	50 mM TRIS pH 7.5, 20 mM KCl, 2 mM Spermine hydrochloride, 30% 2-Methyl-2,4-pentandiol					
K4	**78481**	50 mM HEPES pH 7.0, 20 mM KCl, 5 mM Manganese chloride, 35% 2-Methyl-2,4-pentandiol					
K5	**52239**	50 mM Cacodylate pH 6.5, 1 mM Barium chloride, 20 mM Ammonium chloride, 30% MPD					
K6	**73384**	50 mM Cacodylate pH 6.5, 20 mM NaCl, 1 mM Cadmium chloride, 35% 2-Methyl-2,4-pentandiol					
K7	**97066**	50 mM HEPES pH 7.0, 20 mM NaCl, 5 mM Zinc chloride, 30% 2-Methyl-2,4-pentandiol					
K8	**97068**	50 mM Cacodylate pH 6.5, 15% Isopropanol					
K9	**97067**	20 mM NaCl, 5 mM $MgCl_2$, 15% Isopropanol					
K10	**97069**	50 mM TRIS pH 7.5, 5 mM Cadmium chloride, 2 mM Spermin hydrochloride, 15% Isopropanol					
K11	**69408**	50 mM HEPES pH 7.0, 20 mM Ammonium chloride, 30 % PEG 400					
K12	**74094**	50 mM Cacodylate pH 6.5, 20 mM NaCl, 5 mM $MgCl_2$, 2 mM Spermine hydrochloride, 35 % PEG 400					
K13	**44789**	50 mM TRIS pH 7.5, 20 mM NaCl, 5 mM $MgCl_2$, 35 % PEG 400					
K14	**5336**	50 mM Cacodylate pH 6.5, 20 mM KCl, 1 mM Cadmium chloride, 35 % PEG 400					
K15	**73942**	50 mM HEPES pH 7.0, 20 mM Ammonium chloride, 5 mM $MgCl_2$, 35 % PEG 600					
K16	**68121**	50 mM Cacodylate pH 6.5, 20 mM NaCl, 5 mM $MgCl_2$, 30 % PEG 1000					
K17	**73078**	50 mM Cacodylate pH 6.5, 5 mM $MgCl_2$, 5 mM Spermine hydrochloride, 35 % PEG 4000					
K18	**79993**	50 mM TRIS pH 7.5, 20 mM NaCl, 35 % PEG 8000					
K19	**79182**	50 mM HEPES pH 7.0, 20 mM KCl, 5 mM $MgCl_2$, 1.2 M Ammonium sulfate					
K20	**69566**	50 mM Cacodylate pH 6.5, 20 mM NaCl, 1.2 M Ammonium sulfate					
K21	**52266**	50 mM Cacodylate pH 6.5, 20 mM KCl, 5 mM $MgCl_2$, 1.2 M Sodium acetate					
K22	**41341**	50 mM HEPES pH 7.0, 1 mM Barium chloride, 1.2 M Sodium acetate					
K23	**67627**	50 mM HEPES pH 7.0, 20 mM Sodium chloride, 40 % tert-Butanol					
K24	**92448**	5 mM $MgCl_2$, 2 mM Spermine hydrochloride, 15 % Acetone					
K25	**68115**	50 mM Cacodylate pH 6.5, 20 mM Lithium chloride, 5 mM $MgCl_2$, 35 % 2-Methyl-2,4-pentandiol					
K26	**92102**	50mM Cacodylate pH 6.0, 5 mM Manganese chloride, 1 mM Samarium(III)-chlorid, 5 mM Argininamide, 30 % 2-Methyl-2,4-pentandiol					
K27	**16953**	50 mM TRIS pH 8.0, 20 mM NaCl, 5 mM Zinc chloride, 30 % 2-Methyl-2,4-pentandiol					
K28	**39593**	50 mM TRIS pH 7.5, 1 mM Ruthenium(III)-chloride, 30 % 2-Methyl-2,4-pentandiol					
K29	**53884**	50 mM HEPES pH 7.0, 1 mM Cobalthexamine chloride 30 %, 5 mM Argininamide, 2-Methyl-2,4-pentandiol, 10 % PEG 1000					
K30	**05638**	50 mM Cacodylate pH 6.0, 20 mM Ammonium chloride, 2 mM Putrescine hydrochloride, 30 % 2-Methyl-2,4-pentandiol, 10 % PEG 400					
K31	**05092**	50 mM TRIS pH 8.0, 20 mM KCl, 5 mM $MgCl_2$, 30 % 2-Methyl-2,4-pentandiol, 5 % Isopropanol					
K32	**05630**	50 mM Cacodylate pH 6.0, 1 mM Barium chloride, 20 % Ethanol					
K33	**06391**	50 mM TRIS pH 8.0, 20 mM Ammonium chloride, 1 mM Samarium(III)-chlorid, 20 % Ethanol					
K34	**05639**	50 mM Cacodylate pH 6.0, 20 mM Argininamid, 1 mM Bariumchlorid, 2 mM Spermidine hydrochloride 30 % PEG 400					
K35	**05673**	50 mM Cacodylate pH 6.5, 20 mM KCl, 5 mM Calcium chloride, 30 % PEG 400					
K36	**02969**	50 mM HEPES pH 7.0, 1 mM Cobalthexamine chloride, 35 % PEG 400					
K37	**03268**	50 mM TRIS pH 8.0, 20 mM NaCl, 1 mM Ruthenium(III)-chloride, 35 % PEG 400					
K38	**06531**	50 mM Cacodylate pH 6.5, 20 mM Lithium chloride, 5 mM Cobalt(II)-chloride, 35 % PEG 600					
K39	**11076**	1 mM Cobalthexamine chloride, 2 mM Putrescin hydrochloride, 35 % PEG 600					
K40	**40879**	50 mM Cacodylate pH 6.0, 20 mM Argininamide, 1 mM Cadmium chloride, 30 % PEG 1000					
K41	**42652**	50 mM Cacodylate pH 6.0, 1 mM Bariu mchloride, 1 mM Samarium(III)-chloride, 35 % PEG 4000					
K42	**22664**	50 mM TRIS pH 8.0, 20 mM NaCl, 1 mM Ruthenium(III)-chloride, 35 % PEG 8000					
K43	**40337**	50 mM HEPES pH 7.0, 5 mM Zinc chloride, 35 % PEG 8000					
K44	**57924**	50 mM Cacodylate pH 6.5, 5 mM Calcium chloride, 2 mM Spermidine hydrochloride, 20 % Dioxane					
K45	**50241**	50 mM Cacodylate pH 6.0, 20 mM Lithium chloride, 5 mM Cobalt(II)-chloride, 20 % Dioxane					
K46	**07781**	50 mM TRIS pH 7.5, 20 mM Ammoniumchlorid, 20 % Dioxane					
K47	**36419**	50 mM HEPES pH 7.0, 1 mM Cadmiumchlorid, 2 mM Spermine hydrochloride, 40 % tert-Butanol					
K48	**41718**	50 mM TRIS pH 7.5, 20 mM KCl, 1 mM Barium chloride, 15 % Acetone					

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