

Application

Select the appropriate protein concentration for crystallization screens, particularly Crystal Screen™ and Crystal Screen 2™.

Features

Conserve protein, optimize protein concentration prior to initial screens, provide insight to sample homogeneity.

Description

The PCT™ (Pre-Crystallization Test) is used to determine the appropriate protein concentration for crystallization screening. Sample concentration is a significant crystallization variable. Samples too concentrated can result in amorphous precipitate, while too dilute samples can result in clear drops. Precipitate and clear drops are typical crystallization screen results for reagent conditions which do not promote crystallization and are part of every crystallization screen. However, by optimizing protein concentration for the screen, the number of clear and precipitate results can often be reduced, which in turn results in more efficient sample utilization while at the same time enhancing the chances for crystallization. PCT can minimize or prevent situations where a screen results in an over abundance of precipitate or clear drops.

Introduction

The PCT kit contains 4 unique, preformulated, sterile filtered reagents used to evaluate protein concentration for crystallization screening. Initially, the sample protein is mixed with two of the reagents to determine if the protein concentration is appropriate for crystallization screening. If the protein is very sensitive to salt and polymer concentration, based on initial PCT results, the protein may be evaluated using a second set of PCT reagents. PCT results will then provide insight to either the appropriate sample concentration or indicate that other diagnostic testing such as native gel electrophoresis or dynamic light scattering should be performed to demonstrate sample homogeneity appropriate for crystallization.

Supplied in PCT

- PCT Reagent A1, A2, B1, B2
- Siliconized Glass Cover Slides
- VDX™ Plate with Sealant (HR2-142 kit only)

You Will Also Need Access To:

- Pipet(s)/tips for dispensing 0.05-1 microliter of sample and 500-1,000 microliter of reagent
- Light microscope

Sample Requirements

The sample should not contain phosphate, borate or carbonate buffers. Typical sample concentration for crystallization ranges from less than 5 mg/ml to more than 100 mg/ml but averages 15 mg/ml. PCT will assist in determining the appropriate sample concentration for crystallization screening so there is no required sample concentration for using PCT. Simply concentrate the sample to

a level reasonable for crystallization (5 to 20 mg/ml), in a sample buffer which promotes sample stability and homogeneity.

Instructions (1-12)

1. Pipet 0.5 to 1.0 ml PCT Reagent A1 into reservoir A1 of the VDX Plate with sealant. Pipet 0.5 to 1.0 milliliter of PCT reagent A2 into reservoir A2 of a VDX Plate with sealant.
2. Pipet between 0.05 to 1 microliter of protein sample onto the center of a single glass cover slide.
3. Pipet a volume equal to that used in step 2, of PCT Reagent A1 from reservoir A1 into the sample drop on the siliconized cover slide. Do not mix the drop.
4. Invert the cover slide with the drop over reservoir A1 and seal.
5. Repeat steps 2 through 4 for reagent and reservoir A2.
6. Wait 30 minutes.
7. After 30 minutes, view the two drops using a light microscope with magnification between 20 and 100x. Compare the results to those in Figures 1 and 2. The ideal drop should have a microcrystalline or light granular precipitate throughout the drop. Compare your results to Table 1 and proceed as suggested under Action in Table 1. (See page 2 for instruction number 8).

Table 1

PCT Results and Recommended Action

PCT Reagent A1/B1 Results	PCT Reagent A2/B2 Results	Recommended Action
Heavy Amorphous Precipitate	Heavy Amorphous Precipitate	Dilute sample 1:1, repeat step 1-7
Clear	Clear	Concentrate sample to half the original volume, repeat steps 1-7
Light granular precipitate	Clear	Perform Screen
Clear	Light granular precipitate	Perform Screen
Heavy Amorphous Precipitate	Light granular precipitate	Perform Screen
Heavy Amorphous Precipitate	Clear	Perform PCT with B1 & B2 / perform diagnostic testing
Clear	Heavy Amorphous Precipitate	Perform PCT with B1 & B2 / perform diagnostic testing

Figure 1

Light Precipitate

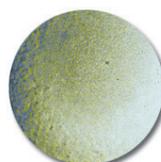
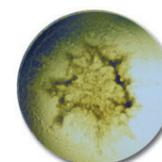


Figure 2

Heavy Amorphous Precipitate



8. Once the appropriate precipitate is observed in the drop using PCT, perform the Crystal Screen or other appropriate crystallization screen using the sample which was successfully evaluated using PCT.

9. If, after diluting the sample to a concentration of 3 mg/ml or less and the drops have a heavy amorphous precipitate, perform PCT with reagent B1 and B2 as outlined in steps 1-7.

10. If, after performing PCT with B1 and B2, the drops have a heavy precipitate consider Diagnostic Testing to measure the homogeneity and purity of the sample. Sample heterogeneity due to impurities, oxidation, denatured protein and sample flexibility can lead to heavy precipitate in the PCT. Diagnostic Testing may include one or more of the following: Denaturing gel electrophoresis may be performed to measure sample purity. Native gel electrophoresis may be performed to measure sample homogeneity. Dynamic light scattering or analytical size exclusion chromatography may be used to measure sample homogeneity. Sample impurity and/or heterogeneity can prevent crystallization. Sample purification (chromatographic), sample modification (site directed mutagenesis), or sample manipulation (use of detergents, additives) should be evaluated in an effort to pursue sample homogeneity.

11. In rare occasions, the drop will first show microcrystalline precipitation after the initial 30 minute incubation, but upon longer incubation will show phase separation. This typically indicates the need for higher protein concentration.

12. For the best PCT data, it is best to wait 24 hours to analyze the status of the drop. Most of us do not have the patience or time to wait 24 hours and only wait 30 minutes.

** The PCT method was developed by Dr. Jarmila Jancarik at the laboratory of Professor Sung-Hou Kim in the Department of Chemistry at the University of California Berkeley.*

PCT Formulation

- Reagent A1 - 0.1 M TRIS hydrochloride pH 8.5, 2.0 M Ammonium sulfate
- Reagent B1 - 0.1 M TRIS hydrochloride pH 8.5, 1.0 M Ammonium sulfate
- Reagent A2 - 0.1 M TRIS hydrochloride pH 8.5,
0.2 M Magnesium chloride hexahydrate,
30% w/v Polyethylene glycol 4,000
- Reagent B2 - 0.1 M TRIS hydrochloride pH 8.5,
0.2 M Magnesium chloride hexahydrate,
15% w/v Polyethylene glycol 4,000

Recommended Optimize™ Reagents

- HR2-727** 1.0 M TRIS hydrochloride, 100 ml
(titrated with NaOH, HR2-583)
- HR2-237** StockOptions™ Tris Hydrochloride Buffer Kit pH 7.0 - 9.0,
(titrated with NaOH, HR2-583)
- HR2-541** 3.5 M Ammonium sulfate, 200 ml
- HR2-559** 2.0 M Magnesium chloride hexahydrate, 100 ml
- HR2-529** 50% w/v Polyethylene glycol 4,000, 200 ml

Related Products

- HR2-140** PCT Kit - 4 unique reagents & 1 pack of slides
- HR2-142** PCT Kit (with plates) - 4 unique reagents, 1 pack of slides,
& 5 VDX Plates with sealant

Technical Support

Inquiries regarding PCT, interpretation of results, and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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