

## Crystallization Screen for Biological Macromolecules

### User Guide

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#### Application

Crystallization screen for biological macromolecules.

Silver Bullets Bio™ can be used as a primary crystallization screen; as a secondary or orthogonal screen to produce crystals when traditional screens are not successful; as an optimization screen in conjunction with preliminary crystallization conditions; as a methodology to uncover different crystal forms; and to produce intramolecular interactions that may stabilize a macromolecules conformation and promote crystallization.

#### Features

Screens a portfolio of small molecules for their ability to establish stabilizing, intermolecular, hydrogen bonding, hydrophobic and electrostatic interactions which could promote stability, lattice formation, and crystallization.

- Small organic molecules, organic salts, and organic acids
- Biologically active molecules, co-factors, and ligands
- Amino acids, peptides, nucleotides, drugs, and carbohydrates
- Biochemical pathway intermediates

#### General Description

Silver Bullets Bio is a kit of 96 solutions designed to provide a rapid screening method for the crystallization of biological macromolecules by employing an alternative strategy using small molecules to promote lattice formation and crystallization. Volume of reagent: 250 microliters. The solutions are compatible with hanging and sitting drop vapor diffusion, microbatch and free interface diffusion crystallization methods. Silver Bullets Bio solutions are designed for use with the Silver Bullets PEG/Tacsimate Crystallization Reagent but may also be used with other crystallization reagents.

#### Definitions

**Sample** refers to the protein, nucleic acid, peptide or other biological macromolecule targeted for crystallization.

\* The Sample only appears in the crystallization drop.

**Silver Bullets Bio** refers to the 96 solutions formulated using small molecules or macromolecular digests and HEPES sodium pH 6.8 buffer.

\* The Silver Bullets Bio are only added to the crystallization drop, not the reservoir solution.

**Crystallization Reagent** refers to the solution in the reservoir and is sometimes called the precipitant, crystallant, dehydrant, or well solution.

\* The Crystallization Reagent is added to both the reagent well (reservoir) and the crystallization drop.

#### Sample Preparation

The macromolecular sample should be homogenous, as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or micro-filtration prior to use.

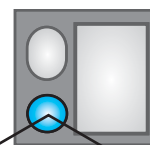
The recommended sample concentration is 5 to 25 mg/ml in dilute buffer (10 to 25 mM). The sample should be free of any unnecessary additives to best observe the effects of the Silver Bullets Bio. Theoretically, the initial screen should be performed with a sample which has been dialyzed against dilute buffer (such as 25 mM HEPES sodium pH 7.0) although in practice, ligands, ions, reducing agents, or other additives may be present as required by the sample for solubility, stability, or activity.

The sample solution should contain the minimum number of chemicals required to stabilize the sample's purity, homogeneity, and activity. An unstable sample is less likely to form reproducible crystals.

#### Drop Setup for Silver Bullets Bio

Recommended drop order and drop ratio listed in Figure 1 below.

Figure 1.  
Intelli-Plate drop well



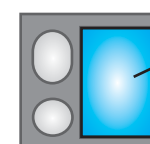
Drop Order	Drop Ratio	Drop Size Example
1. Sample	2	400 nanoliters
2. Silver Bullets Bio	1	200 nanoliters
3. Crystallization Reagent	1	200 nanoliters

Silver Bullets Bio 1-96 (A1-H12) were designed to be used with the Silver Bullets PEG/Tacsimate Crystallization Reagents 1-96 (A1-H12), but they may also be used with other Crystallization Reagents. See Related Product section on page 4.

#### Crystallization Reagent Setup for Silver Bullets Bio

Pipette 90 microliters (or the plate maker's recommended reservoir volume) of PEG/Tacsimate Crystallization Reagent into each of the 96 reservoirs.

Figure 2.  
Intelli-Plate reagent well



90 microliters of  
Crystallization Reagent

PEG/Tacsimate Crystallization Reagents 1-96 (A1-H12) should be dispensed into the crystallization plate reservoirs 1-96 (A1-H12) so the reagents are combined correctly with the intended Silver Bullets 1-96 (A1-H12).

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#### Using Other Crystallization Reagents with Silver Bullets Bio

Silver Bullets Bio may be used with crystallization reagents other than the (Silver Bullets) PEG/Tacsimate Crystallization Reagents. For example, let's say one has used a screen and found one or more crystallization reagents that produce crystals, or any other promising outcome that might be pursued. Or one has used another crystallization screen and found one or more crystallization reagents that produce an interesting or promising result. Or perhaps, there is evidence that suggests a sample may have a better chance for crystallization in a crystallization reagent other than PEG/Tacsimate or other (Silver Bullets) Crystallization Reagent. One could then substitute any of these crystallization reagents in both the reagent well and in the crystallization drop in conjunction with the Silver Bullets Bio. However, to identify Silver Bullets Bio and crystallization reagent mixtures which might produce salt crystals, one should perform a control experiment (see Control Experiment).

**Tip:** To try a reagent from a Hampton Research screen, order the individual reagent from the Custom Shop and run the reagent with the Silver Bullets Bio.

#### Silver Bullets Bio Formulation

Silver Bullets are % w/v solution in ASTM Type I 18.2 MegaOhm reagent water and HEPES sodium pH 6.8 buffer. Once the chemicals are added to the buffer and water the solution is warmed to 40 degrees Celsius to enhance chemical solubility, followed by incubation at 25 degrees Celsius. The pH of each reagent is adjusted to pH 6.8 at 25 degrees Celsius using Hydrochloric acid or Sodium hydroxide. The resulting solution is centrifuged to pellet any precipitate and the supernatant is filtered using a 0.22 micron sterile filter into a sterile container. HEPES sodium buffer CAS [75277-39-3] is titrated to pH 6.8 using Hydrochloric acid. Refer to the Excel document entitled "Silver Bullets Bio Specifications" for complete chemical names, synonyms, molecular formula, Mr, CAS, EC, structure, Beilstein Number and RTECS information. Each Silver Bullets Bio solution is available separately through the Hampton Research Custom Shop™.

#### Storing, Handling, & Use

Silver Bullets Bio should be stored between -20 and 25 degrees Celsius. If the solutions have been below 25 degrees Celsius, or if precipitate is present, warm the reagents to 40 degrees Celsius for 1 to 4 hours, mix well and then allow the solutions to equilibrate to 25 degrees Celsius.

Centrifuge the 96 well plate that contains the Silver Bullets Bio at 1,000 RPM for 10 minutes to pellet any remaining precipitate. The Solutions are now ready for use.

The block is thermal sealed with a polypropylene/aluminum film. The film should be removed when the block is at a temperature of 25 degrees Celsius. Removing the film with the block temperature below 25 degrees Celsius can

delaminate or shred the film. After use, seal the block using AlumaSeal II film (HR8-069), or for best results the block should be sealed using a thermal sealer.

#### Reagent Solubility

Silver Bullets Bio are formulated at or near the saturation of each chemical at pH 6.8 in 0.02 M HEPES sodium buffer. The solubility is temperature dependent, pH dependent and also depends on the presence of other chemicals. Storage or exposure of the reagents to temperatures below 25 degrees Celsius will precipitate some of the Silver Bullets Bio. This is normal. When this happens, the reagents should be warmed to 40 degrees Celsius for 1 to 4 hours and mixed to promote solubility.

Allow the Silver Bullets Bio to re-equilibrate at 25 degrees Celsius and centrifuge the block before use.

Centrifuge the block at 1,000 rpm for 10 minutes to sediment any remaining precipitate. When setting the crystallization experiment, avoid touching the aspirating pipette tips to the bottom of the reagent well to avoid clogging tips or suspending the precipitate.

The chemicals in Silver Bullets Bio are capable of forming small molecule salt crystals (false positives). Salt crystals may form due to:

- Changes in pH
- A lowering of Temperature
- An increase in the relative supersaturation of the reagent
- The presence of polyvalent ions
- The presence of salts \* (including but not limited to phosphate, borate and carbonate) or evaporation.

To minimize evaporation from the open block, place a Plate Lid (HR3-084) on top of the opened block when it is not being stored or in active use. When viewing crystallization experiment drops, one should be aware of the possibility of salt crystals. To test for the presence of salt crystals, it is suggested one set a control experiment (see Control Experiment on page 3).

#### Silver Bullets Bio & pH

Silver Bullets Bio are buffered in 0.02 M HEPES sodium pH 6.8 but can be screened at different pH levels in the presence of higher concentration (0.1 M) buffers. Since the recommended drop ratio is 1 part Silver Bullets Bio: 1 part Crystallization Reagent : 2 parts Sample, a 0.1 M Crystallization Reagent buffer in the drop and reservoir will be of sufficient concentration to determine and control the final pH in the experiment.

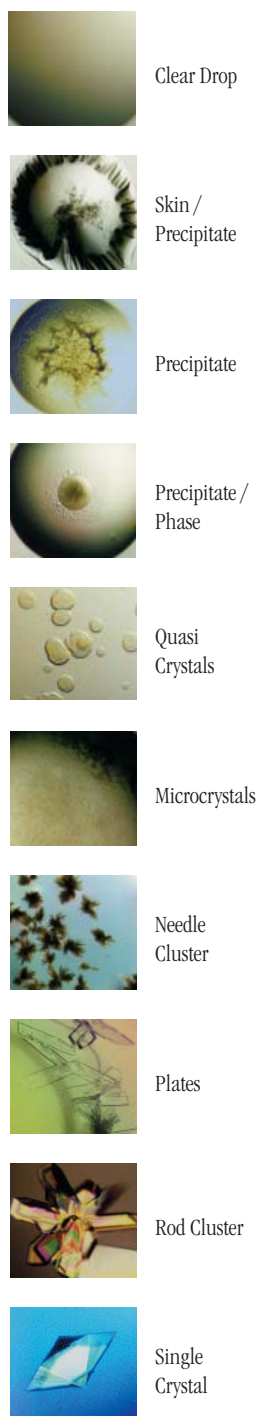
Silver Bullets Bio have been tested successfully for use between pH 5.5-8.0. While it may be possible to use the Silver Bullets Bio below pH 5.5 and above 8.0, this application has not been validated. For a more comprehensive Silver Bullets Bio screen, screen the Silver Bullets Bio at pH 5.8, 6.8, and 7.8.

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**Figure 3.**  
Typical observations in a  
crystallization experiment



### Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week thereafter until the drops dry out. Records should indicate whether the drop is clear, contains precipitate, and or crystals. Some find it helpful to describe the drop contents using magnitude and descriptive terms. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Unknown = 3, Crystal = 4 or other format) or even a color based scoring scheme. Figure 3 (left side of page) shows typical examples of what one might observe in a crystallization experiment.

### Interpreting the Drop

Most of the chemicals in the Silver Bullets Bio solutions are used more than once, and some are used up to seven times in the kit. The duplication can be used to extrapolate which chemical may be the crystal's silver bullet.

For example, the appearance of crystals in drops 3, 5, and 15 might indicate that trans-Cinnamic acid is the silver bullet since trans-Cinnamic acid is a common chemical to each of these solutions.

In Silver Bullets Bio solutions where the chemical is used only once, such as Taurine in reagent 4, one should initially perform optimization using a complete Silver Bullets Bio solution 4 as it may not be necessary to screen individual Silver Bullets Bio chemicals to optimize the condition.

### Optimization

When optimizing a crystal produced from a Silver Bullets Bio solution, one should pursue strategies, methods and techniques typically used for crystal optimization. This would include varying the pH, the crystallization reagent composition and the crystallization reagent concentration. One should also consider the effects of temperature between 4 and 30 degrees Celsius; Seed from anything crystalline or precipitated outcomes; Vary drop ratios; Further purify or modify the sample. And of course consider evaluating other small molecules which may be required by your sample to produce or improve crystals.

### Control Experiment

A control experiment is used to identify false leads, or salt crystals, where all components are present from the original crystallization experiment, minus the sample. The sample solution, complete with all chemicals, minus the sample should be used in place of the original sample. The presence of crystals in the control drop that appear similar to those in the original crystallization experiment indicates the formation of salt crystals. For best interpretation, the control experiment should be performed using the same crystallization method, with the same device, materials, temperature, and volumes and evaluated on the same time scale and in a manner similar to the original crystallization experiment.

### References

1. Searching for silver bullets: An alternative strategy for crystallizing macromolecules. Alexander McPherson and Bob Cudney. *Journal of Structural Biology* 156 (2006) 387-406.
2. A novel strategy for the crystallization of proteins: X-ray diffraction validation. Steven B. Larson, John S. Day, Robert Cudney, and Alexander McPherson. *Acta Cryst.* (2007) D63, 310-318.
3. A new crystal form of bovine pancreatic RNase A in complex with 2'-deoxyguanosine-5'-monophosphate. S. B. Larson, J. S. Day, R. Cudney and A. McPherson. *Acta Cryst.* (2007). F63, 728-733.
4. Development of an alternative approach to protein crystallization. McPherson, Alexander; Nguyen, Chieniang; Larson, Steven B; Day, John S; Cudney, Bob. *J Struct Funct Genomics*, Volume 8, Number 4, December 2007, 193-198.
5. Progress in the Development of an Alternative Approach to Macromolecular Crystallization. S. B. Larson, J. S. Day, C. Nguyen, R. Cudney, and A. McPherson. *Crystal Growth & Design* 2008 Volume 8, No. 8 3038-3052.

### Technical Support

Inquiries regarding Silver Bullets Bio solution formulation, interpretation of screen results, optimization strategies 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 5:00 p.m. USA Pacific Standard Time.

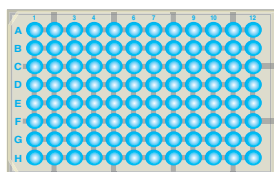
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#### Related Products

Catalog #	Name	Description
HR2-080	Silver Bullets Bio	0.25 ml, tube format
HR2-088	Silver Bullets Bio HT	0.25 ml, block format
HR2-078	Silver Bullets	0.25 ml, tube format
HR2-096	Silver Bullets HT	0.25 ml, block format



Individual Silver Bullets are available through the Hampton Research Custom Shop.  
Please visit [www.hamptonresearch.com](http://www.hamptonresearch.com) for ordering information.

#### Reproducing & Optimization

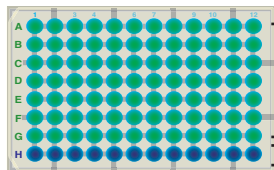
The following are used to reproduce and optimize Crystallization Reagents for use with Silver Bullets.

Catalog #	Name	Description	Used in...
HR2-837	25% w/v Polyethylene glycol 3,350, 0.1 M MES monohydrate pH 5.8	100 ml	A1 - G12 HR2-090
HR2-839	55% v/v Tacsimate pH 6.0, 0.1 M MES monohydrate pH 5.8	100 ml	H1 - H12 HR2-090
HR2-527	50% w/v Polyethylene glycol 3,350	200 ml	HR2-090 (A1 - G12)
HR2-943-07	1.0 M MES monohydrate pH 5.8	185 ml	HR2-090 (A1 - H12)
HR2-827	100% Tacsimate pH 6.0	200 ml	HR2-090 (H1 - H12)
HR2-841	25% w/v Polyethylene glycol 3,350, 0.1 M HEPES sodium pH 6.8	100 ml	A1 - G12 HR2-092
HR2-843	55% v/v Tacsimate pH 7.0, 0.1 M HEPES sodium pH 6.8	100 ml	H1 - H12 HR2-092
HR2-527	50% w/v Polyethylene glycol 3,350	200 ml	HR2-092 (A1 - G12)
HR2-931-01	1.0 M HEPES sodium pH 6.8	185 ml	HR2-092 (A1 - H12)
HR2-755	100% Tacsimate pH 7.0	200 ml	HR2-092 (H1 - H12)
HR2-849	25% w/v Polyethylene glycol 3,350, 0.1 M BIS-TRIS propane pH 7.8	100 ml	A1 - G12 HR2-094
HR2-851	55% v/v Tacsimate pH 8.0, 0.1 M BIS-TRIS propane pH 7.8	100 ml	H1 - H12 HR2-094
HR2-527	50% w/v Polyethylene glycol 3,350	200 ml	HR2-094 (A1 - G12)
HR2-993-16	1.0 M BIS-TRIS propane pH 7.8	185 ml	HR2-094 (A1 - H12)
HR2-829	100% Tacsimate pH 8.0	200 ml	HR2-092 (H1 - H12)

#### Crystallization Reagent - Screening

The following are offered as a 1 milliliter fill in a Deep Well block and are used as the Crystallization Reagent with the Silver Bullets.

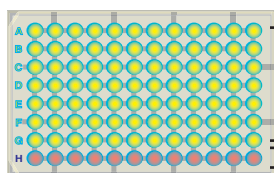
Catalog #	Name	Description
HR2-090	PEG/Tacsimate pH 5.8 Crystallization Reagent for Silver Bullets	1.0 milliliter, Deep Well block



**Reagent A1 - G12**  
25% w/v Polyethylene glycol 3,350,  
0.1 M MES monohydrate pH 5.8

**Reagent H1 - H12**  
55% v/v Tacsimate pH 6.0,  
0.1 M MES monohydrate pH 5.8

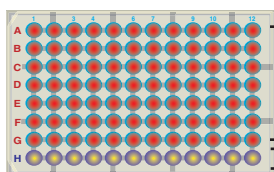
HR2-092	PEG/Tacsimate pH 6.8 Crystallization Reagent for Silver Bullets	1.0 milliliter, Deep Well block
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**Reagent A1 - G12**  
25% w/v Polyethylene glycol 3,350,  
0.1 M HEPES sodium pH 6.8

**Reagent H1 - H12**  
55% v/v Tacsimate pH 7.0,  
0.1 M HEPES sodium pH 6.8

HR2-094	PEG/Tacsimate pH 7.8 Crystallization Reagent for Silver Bullets	1.0 milliliter, Deep Well block
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**Reagent A1 - G12**  
25% w/v Polyethylene glycol 3,350,  
0.1 M BIS-TRIS propane pH 7.8

**Reagent H1 - H12**  
55% v/v Tacsimate pH 8.0,  
0.1 M BIS-TRIS propane pH 7.8

◊ The reagent colors featured in this User Guide are for illustration purposes only.

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