

## Application

Crystallization screen for proteins, peptides, nucleic acids, macromolecular complexes and water-soluble small molecules.

## Formats

Bulk – 4 Kits containing 24 screening solutions each at 10 ml aliquots

HTS – 96 solutions of JBScreen PACT++ delivered in a 96 deep-well-block

HTS S: 1.0 ml per well

HTS L: 1.7 ml per well

## Storage and Expiry

The kit is stable at ambient temperature, however, should be kept at 4°C for long-term storage.

If stored correctly, Jena Bioscience guarantees a shelf life of 12 months.

## Background

**JBScreen PACT++** is a crystallization screen facilitating systematic **pH**, **anion-** and **cation** testing in the presence of polyethylene glycol (PEG) [1].

The 96 unique reagents incorporate three screens in one. For each sub-screen a different PEG concentration is chosen and the PEG concentration is kept constant within each screen.

### 1. PEG/pH Screen

The *PEG/pH Screen* comprises 24 conditions. 4 broad range buffer systems are screened against 25% w/v PEG 1500. Each buffer system is adjusted to 6 distinct pH values, allowing to screen a pH range from pH 4 to pH 9 without changing the chemical nature of the reagent solution [2].

The exact composition of the broad range buffer systems is described in Tab.1.

In contrast to the crystal screen PACT, published on the SPINE website [3], JBScreen PACT++ does not contain cacodylate. According to our philosophy to omit the use of cacodylate, the buffer system PCB (Propionate, Cacodylate, Bis-Tris Propane) was replaced by TBG (Tartrate, Bis-Tris, Glycylglycine), which is described by Newman *et al.* [2], too.

**Table 1.** Broad range buffer systems used in the PEG/pH Screen

| Abbr. | Buffer composition          | Ratio | pH low | pH high |
|-------|-----------------------------|-------|--------|---------|
| SPG   | Succinic Acid               | 2     | 4.0    | 10.0    |
|       | Sodium dihydrogen Phosphate | 7     |        |         |
|       | Glycine                     | 7     |        |         |
| MIB   | Malonic Acid                | 2     | 4.0    | 10.0    |
|       | Imidazole                   | 3     |        |         |
|       | Boric Acid                  | 3     |        |         |
| TBG   | Sodium Tartrate dihydrate   | 3     | 4.0    | 9.0     |
|       | Bis-Tris                    | 2     |        |         |
|       | Glycylglycine               | 2     |        |         |
| MMT   | L-Malic Acid                | 1     | 4.0    | 9.0     |
|       | MES                         | 2     |        |         |
|       | Tris                        | 2     |        |         |

### 2. PEG/Cation Screen

The 24 conditions of the *PEG/Cation Screen* are designed to screen 6 different cations against 20% w/v PEG 6000 at 4 distinct pH values. All cations (Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, Li<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup> and Zn<sup>2+</sup>) have chloride as counter ion. Acetate, MES, HEPES and Tris are the buffers used, adjusted to pH 5, pH 6, pH 7 and pH 8, respectively.

### 3. PEG/Anion Screen

The *PEG/Anion Screen* comprises 48 conditions. 12 different anions, i.e. fluoride, bromide, iodide, thiocyanate, nitrate, formate, acetate, sulphate, tartrate, phosphate, citrate and malonate, are screened against 20% w/v PEG 3350. Either sodium or potassium are present as counter ion.

In order to evaluate the effect of the pH, the 12 anions are screened against PEG using Bis-Tris Propane buffer at pH values of 6.5, 7.5 and 8.5. Furthermore one set of anions is screened without any buffer solution.

Please consult our formulation sheets for the exact composition of **JBScreen PACT++**.

## Instructions

The layout of the screen is designed for setting up a 96 well plate. Pipette the solutions starting with A1 horizontally to A12, then the 2<sup>nd</sup> row from B1 to B12 down to H1 to H12. Then your plate is divided into 3 sub-screens as depicted in Fig. 1.

|   | 1 | 2                   | 3              | 4 | 5                   | 6              | 7 | 8                   | 9 | 10 | 11 | 12 |
|---|---|---------------------|----------------|---|---------------------|----------------|---|---------------------|---|----|----|----|
| A |   |                     |                |   |                     |                |   |                     |   |    |    |    |
| B |   |                     | <b>24 well</b> |   |                     |                |   | <b>24 well</b>      |   |    |    |    |
| C |   | <b>25% PEG 1500</b> |                |   |                     |                |   | <b>20% PEG 6000</b> |   |    |    |    |
| D |   | <b>vs pH</b>        |                |   |                     |                |   | <b>vs Cations</b>   |   |    |    |    |
| E |   |                     |                |   |                     |                |   |                     |   |    |    |    |
| F |   |                     |                |   |                     | <b>24 well</b> |   |                     |   |    |    |    |
| G |   |                     |                |   | <b>20% PEG 3350</b> |                |   |                     |   |    |    |    |
| H |   |                     |                |   | <b>vs Anions</b>    |                |   |                     |   |    |    |    |

**Fig. 1.** JBScreen PACT++ consists of three sub-screens, each containing a different PEG.

### References

- [1] Newman *et al.* (2005) Towards rationalization of crystallization screening for small- to medium-sized academic laboratories: the PACT/JCSC+ strategy. *Acta Cryst. D***61**:1426.
- [2] Newman (2004) Novel buffer systems for macromolecular crystallization. *Acta Cryst. D***60**:610.
- [3] <http://www.spineurope.org>

# Scoring Sheet **JBScreen PACT++**



Name: \_\_\_\_\_ Sample Name: \_\_\_\_\_ Temperature: \_\_\_\_\_

Plate ID: \_\_\_\_\_ Sample Concentration: \_\_\_\_\_ Reservoir Volume: \_\_\_\_\_

Set-up Date: \_\_\_\_\_ Sample Buffer : \_\_\_\_\_ Total Drop Volume: \_\_\_\_\_  $\mu$ l

Observation Date: \_\_\_\_\_ Sample : \_\_\_\_\_  $\mu$ l Reservoir : \_\_\_\_\_  $\mu$ l Additive: \_\_\_\_\_  $\mu$ l

|          | 1  | 2   | 3  | 4   | 5  | 6  | 7  | 8  | 9  | 10  | 11   | 12   |
|----------|--|---|--|---|--|--|--|--|--|---|--|--|
| <b>A</b> | 25% PEG 1500<br>SPG Buffer<br>pH 4                       | 25% PEG 1500<br>SPG Buffer<br>pH 5                        | 25% PEG 1500<br>SPG Buffer<br>pH 6                       | 25% PEG 1500<br>SPG Buffer<br>pH 7                        | 25% PEG 1500<br>SPG Buffer<br>pH 8                                     | 25% PEG 1500<br>SPG Buffer<br>pH 9                           | 20% PEG 6000<br>Acetate pH 5<br>200 mM NaCl                | 20% PEG 6000<br>Acetate pH 5<br>200 mM NH <sub>4</sub> Cl                            | 20% PEG 6000<br>Acetate pH 5<br>200 mM LiCl                    | 20% PEG 6000<br>Acetate pH 5<br>200 mM MgCl <sub>2</sub>                  | 20% PEG 6000<br>Acetate pH 5<br>200 mM CaCl <sub>2</sub> | 20% PEG 6000<br>Acetate pH 5<br>10 mM ZnCl <sub>2</sub>  |
| <b>B</b> | 25% PEG 1500<br>MIB Buffer<br>pH 4                       | 25% PEG 1500<br>MIB Buffer<br>pH 5                        | 25% PEG 1500<br>MIB Buffer<br>pH 6                       | 25% PEG 1500<br>MIB Buffer<br>pH 7                        | 25% PEG 1500<br>MIB Buffer<br>pH 8                                     | 25% PEG 1500<br>MIB Buffer<br>pH 9                           | 20% PEG 6000<br>MES pH 6<br>200 mM NaCl                    | 20% PEG 6000<br>MES pH 6<br>200 mM NH <sub>4</sub> Cl                                | 20% PEG 6000<br>MES pH 6<br>200 mM LiCl                        | 20% PEG 6000<br>MES pH 6<br>200 mM MgCl <sub>2</sub>                      | 20% PEG 6000<br>MES pH 6<br>200 mM CaCl <sub>2</sub>     | 20% PEG 6000<br>MES pH 6<br>10 mM ZnCl <sub>2</sub>      |
| <b>C</b> | 25% PEG 1500<br>TBG Buffer<br>pH 4                       | 25% PEG 1500<br>TBG Buffer<br>pH 5                        | 25% PEG 1500<br>TBG Buffer<br>pH 6                       | 25% PEG 1500<br>TBG Buffer<br>pH 7                        | 25% PEG 1500<br>TBG Buffer<br>pH 8                                     | 25% PEG 1500<br>TBG Buffer<br>pH 9                           | 20% PEG 6000<br>HEPES pH 7<br>200 mM NaCl                  | 20% PEG 6000<br>HEPES pH 7<br>200 mM NH <sub>4</sub> Cl                              | 20% PEG 6000<br>HEPES pH 7<br>200 mM LiCl                      | 20% PEG 6000<br>HEPES pH 7<br>200 mM MgCl <sub>2</sub>                    | 20% PEG 6000<br>HEPES pH 7<br>200 mM CaCl <sub>2</sub>   | 20% PEG 6000<br>HEPES pH 7<br>10 mM ZnCl <sub>2</sub>    |
| <b>D</b> | 25% PEG 1500<br>MMT Buffer<br>pH 4                       | 25% PEG 1500<br>MMT Buffer<br>pH 5                        | 25% PEG 1500<br>MMT Buffer<br>PH 6                       | 25% PEG 1500<br>MMT Buffer<br>pH 7                        | 25% PEG 1500<br>MMT Buffer<br>pH 8                                     | 25% PEG 1500<br>MMT Buffer<br>pH 9                           | 20% PEG 6000<br>Tris pH 8<br>200 mM NaCl                   | 20% PEG 6000<br>Tris pH 8<br>200 mM NH <sub>4</sub> Cl                               | 20% PEG 6000<br>Tris pH 8<br>200 mM LiCl                       | 20% PEG 6000<br>Tris pH 8<br>200 mM MgCl <sub>2</sub>                     | 20% PEG 6000<br>Tris pH 8<br>200 mM CaCl <sub>2</sub>    | 20% PEG 6000<br>Tris pH 8<br>10 mM ZnCl <sub>2</sub>     |
| <b>E</b> | 20% PEG 3350<br>200 mM NaF                               | 20% PEG 3350<br>200 mM NaBr                               | 20% PEG 3350<br>200 mM NaI                               | 20% PEG 3350<br>200 mM KSCN                               | 20% PEG 3350<br>200 mM NaNO <sub>3</sub>                               | 20% PEG 3350<br>200 mM NaForm.                               | 20% PEG 3350<br>200 mM NaAc.                               | 20% PEG 3350<br>200 mM Na <sub>2</sub> SO <sub>4</sub>                               | 20% PEG 3350<br>200 mM Na/K Tar.                               | 20% PEG 3350<br>200 mM Na/K PO <sub>4</sub>                               | 20% PEG 3350<br>NaCitrate                                | 20% PEG 3350<br>NaMalonate                               |
| <b>F</b> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM NaF | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM NaBr | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM NaI | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM KSCN | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM NaNO <sub>3</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM NaForm. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM NaAc. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM Na <sub>2</sub> SO <sub>4</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM Na/K Tar. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>200 mM Na/K PO <sub>4</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>NaCitrate  | 20% PEG 3350<br>Bis-Tris Propane<br>pH 6.5<br>NaMalonate |
| <b>G</b> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM NaF | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM NaBr | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM NaI | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM KSCN | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM NaNO <sub>3</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM NaForm. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM NaAc. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM Na <sub>2</sub> SO <sub>4</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM Na/K Tar. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>200 mM Na/K PO <sub>4</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>NaCitrate  | 20% PEG 3350<br>Bis-Tris Propane<br>pH 7.5<br>NaMalonate |
| <b>H</b> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM NaF | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM NaBr | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM NaI | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM KSCN | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM NaNO <sub>3</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM NaForm. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM NaAc. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM Na <sub>2</sub> SO <sub>4</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM Na/K Tar. | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>200 mM Na/K PO <sub>4</sub> | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>NaCitrate  | 20% PEG 3350<br>Bis-Tris Propane<br>pH 8.5<br>NaMalonate |

For questions and inquires please contact [xtals@jenabioscience.com](mailto:xtals@jenabioscience.com)