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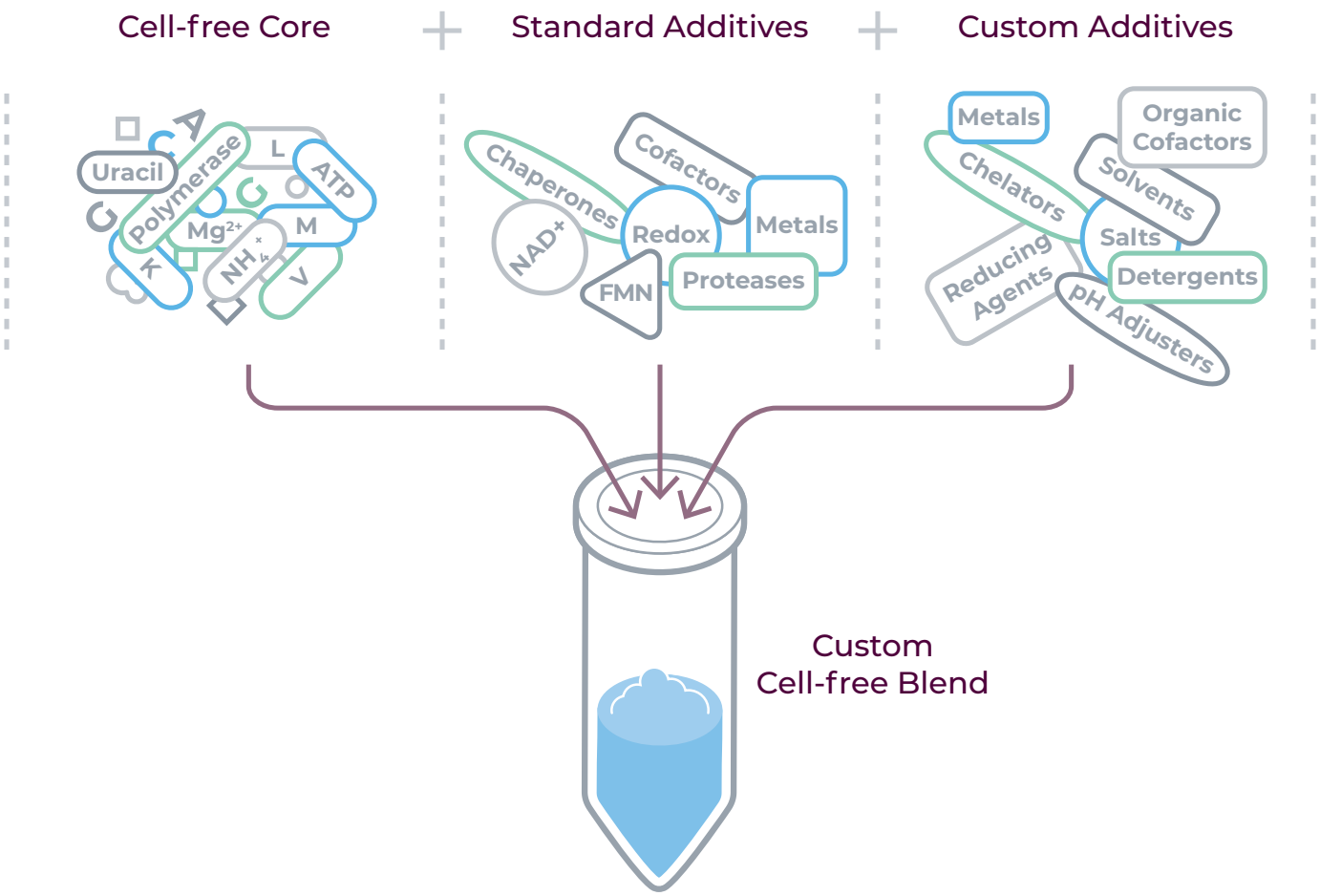
Custom Additives: tailoring protein expression for your research needs

Unlock the full potential of the eProtein Discovery™ system with Custom Additives

The Custom Additives capability empowers users to customize Cell-free Blends with additives of their own choice. This increases the flexibility for optimizing protein expression, folding and purification, by using user-chosen additives that are better tailored for their specific protein requirements while maintaining the automation and reproducibility provided by the eProtein Discovery™ system.



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Why use Custom Additives?

- **Flexibility:**
  Tailor protein expression conditions to suit your unique protein targets.
- **Scalability:**
  Achieve consistent results across different scales of production.
- **Optimization:**
  Address specific challenges like protein folding, aggregation, and activity.
- **Compatibility:**
  Verified on our system for seamless integration of up to 16 user-specified additives per run.

Category	Importance in protein science
Detergents and surfactants	Solubilizing membrane proteins, preventing aggregation, and maintaining protein stability.
Protein storage buffers	Useful for preserving protein stability and activity during storage, preventing degradation and aggregation.
Salts and ionic strength modifiers	Adjusting ionic strength, stabilizing proteins, and influencing solubility.
Cofactors and enzyme activators	Essential cofactors or enzymatic activators for protein functionality.
Buffers and pH stabilizers	Maintaining pH stability and ionic strength, critical for optimal protein folding and activity.
Stabilizers and cryoprotectants	Stabilizing proteins during expression, purification, and storage.
Reducing agents	Preventing oxidation, maintaining reducing environments, and stabilizing disulfide bonds.
Organic solvents	Improving protein solubility, acting as cryoprotectants, or aiding in ligand binding studies.
Chelating agents	Binding metal ions, preventing metal-catalyzed oxidation, and assisting protein purification.
Nanodiscs	Membrane scaffold proteins and lipids create a native-like environment that stabilizes membrane proteins, improving yield, folding, and function.

Preparation of Custom Additive Cell-free Blends for use with eProtein Discovery

1. Prepare custom additive solutions as desired, ensuring that no component in the final formulation of the additive solution exceeds the concentration of the identical, or closely similar, compound listed in the compatibility table.

2. All custom additive solutions should be prepared and diluted in MilliQ water, unless otherwise noted.

3. Use the custom additive solution prepared in step 1 to prepare a customized Cell-free Blend by dispensing the appropriate volume of additive into the core reagent. Custom additives can be used as single or double additives, unless stated otherwise. For a reminder of how to prepare Cell-free Blends, please refer to the eProtein Discovery user guide.

4. Once prepared, use the customized Cell-free Blends to prepare a transfer plate as normal, simply replacing the standard Cell-free Blend wells, and proceed with the eProtein Discovery screen as normal.

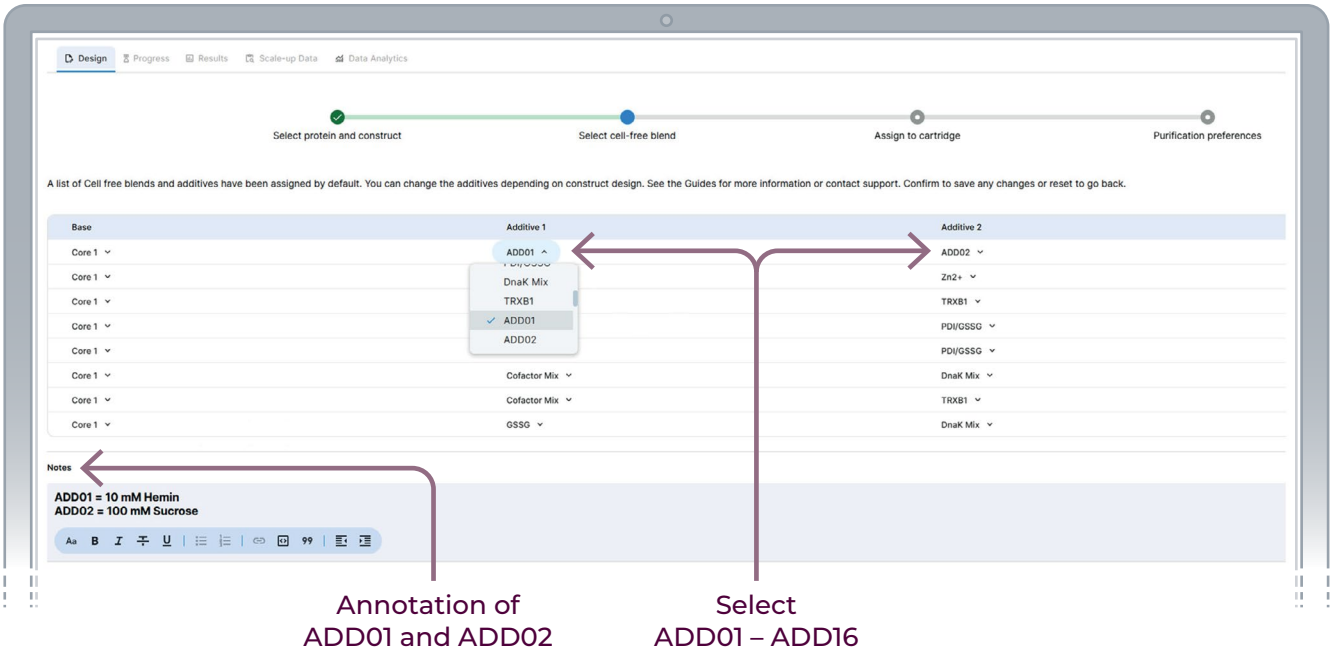
Input of Custom Additives in eProtein Discovery Cloud Software

- The ability to select and annotate up to 16 custom additives per run has been provided in the eProtein Discovery Cloud Software.

- Users should proceed to input their proteins-of-interest (POIs) as per the user guide. During the 'Experiment Design' phase, users will 'Select protein and construct' as usual.

- During 'Select Cell-free Blend' phase, users now have the option to select up to 16 custom additives (called ADD01 - ADD16) from the dropdown menus under 'Additive 1' and 'Additive 2' (please see screenshot below).

- Users should also annotate within the 'Notes' section what the additives and their concentrations are along with any information they might find useful so that this customization information is linked to the screening run.



Chemical compatibility list with maximum concentrations

This chemical compatibility list is designed to guide you on the maximum concentrations compatible with expression, purification, and droplet movement on the eProtein Discovery Cartridge. Some chemicals in the list serve as solvents for solubilizing specific chemical additives. To improve the likelihood of success and minimize the risk of run failures, we recommend using concentrations below the stated maximums and performance cannot be guaranteed if these maximum values are exceeded. More details are provided in the 'Disclaimer' section.

Additive(s)	Max. stock concentration[1]
Buffers and pH modifiers	
Tris pH 7.5 - 9.0	50 mM
HEPES pH 7.3 - 8.0	500 mM
Sodium phosphate pH 7.5	50 mM
Sodium hydroxide (NaOH)	100 mM
Hydrochloric acid (HCl)	30 mM
Buffers with pH < 6	Not compatible
Buffers with pH > 9	Not compatible
Salts and ionic strength modifiers	
Sodium chloride (NaCl)	350 mM
Potassium chloride (KCl)	350 mM
Ammonium sulfate	350 mM
Reducing agents	
Dithiothreitol (DTT)	65 mM
Tris(2-carboxyethyl)phosphine (TCEP)	30 mM
L-Glutathione reduced (GSH)	10 mM
Stabilisers and cryoprotectants	
Sucrose	500 mM
Glycerol	10%
PEG8000	2%
Dextran	5%
Bovine serum albumin (BSA)	15 mg/mL
Betaine monohydrate	2.5 M
Organic solvents	
Dimethyl sulfoxide (DMSO)	10%
Dimethyformamide (DMF)	10%
Ethanol	3%
Chelating agents	
EDTA	10 mM
Protein purification elution buffer components	
Imidazol	250 mM
Biotin	Not compatible

Additive(s)	Max. stock concentration[1]
Metal ion and organic cofactors	
ATP[2] Magnesium chloride[2]	10 mM 10 mM
Hemin[3]	65 µM
Iron (II) chloride	1 mM
Ascorbic acid	5 mM
Flavin mononucleotide (FMN)[4]	130 µM
Thiamine pyrophosphate (TPP)	10 mM
Cobalt chloride	0.60 mM
Biopterin	0.60 mM
Nicotinamide adenine dinucleotide phosphate (NADPH)	10 mM
Ubiquinone	Not compatible
Cu^{+2}	Not compatible
Protein storage buffers	
Phosphate buffered saline (PBS)	1X
Tris pH 7.5 Sodium chloride (NaCl) Glycerol Dithiothreitol (DTT)	50 mM 250 mM 10% 1 mM
HEPES pH 7.3 Potassium chloride (KCl) Glycerol Dithiothreitol (DTT)	50 mM 250 mM 10% 1 mM
HEPES pH 7.3 Potassium chloride (KCl) Glycerol Dithiothreitol (DTT) Imidazole	50 mM 100 mM 10% 1 mM 250 mM
Detergents and surfactants	
Lauryl maltose neopentyl glycol (LMNG)	5%
Lauryl maltose neopentyl glycol (LMNG) Cholesteryl hemisuccinate (CHS)	5% 0.5%
Glyco-diosgenin (GDN)	1.6%
Digitonin	6.6%
Octylglucopyranoside (OG)	6.2 mM
Brij®-35	6.6%
Brij®-58	6.6%
Tween®-20 (polysorbate 20)	6.6%
Tween®-80 (polysorbate 80)	6.6%
Fos-choline-12 (MAPCHO®-12)	5.4 mM
Fos-choline-14 (MAPCHO®-14)	0.3 mM
CHAPS	20 mM
Lauryldimethylamine-N-oxide (LDAO)	1.2 mM
Sodium deoxycholate	0.9 mM

Additive(s)	Max. stock concentration[1]
Sodium cholate hydrate	4.6 mM
Dodecyltrimethylammonium chloride	1 mM
Benzyldodecyldimethylammonium bromide	0.1 mM
Non detergent sulfobetaines 195 (NDSB-195)	500 mM
BIG CHAP	Not compatible
N-lauroylsarcosine	Not compatible
Cetyltrimethylammonium bromide (CTAB)	Not compatible
n-dodecyl-β-D-maltoside (DDM)	Not compatible
Triton® X-100	Not compatible
Nanodiscs[5]	
MSP1D1dH5-DMPG	500 µM
MSP1E3D1 -DMPG	500 µM
MSP2N2-DMPG	500 µM
MSP1D1dH5-POPC	500 µM
MSP1E3D1 -POPC	500 µM
MSP2N2-POPC	500 µM
MSP1D1dH5-DMPC	500 µM
MSP1E3D1 -DMPC	500 µM
MSP2N2-DMPC	500 µM

1. Listed are the concentrations that should not be exceeded when preparing custom additive stock solutions. These will then get diluted to tolerated final concentrations when formulated into Cell-free Blends, as described in the eProtein Discovery User guide. Note that in the final reaction on-cartridge, custom additive stock solutions will be diluted 13.4X or 6.7X for single or double additives, respectively.

2. ATP and magnesium should only be used in combination.

3. If hemin is chosen as a custom additive, it should be included in all Cell-free Blends in a run. For detailed instructions on how to prepare and use hemin as a custom additive, please reach out to Nuclera Technical Support.

4. FMN increases background fluorescence of expression droplets. If FMN is chosen as a custom additive, FMN should be included in all Cell-free Blends in a run, to normalize for this increased fluorescent background.

5. We recommend adding only 2 µL of the nanodisc stock solution to the Cell-free Blend, with the remaining 2 µL consisting of selected standard additives or buffer. The nanodiscs tested were obtained from Cube Biotech. Membrane scaffold proteins derived from human, mouse, and rat, both untagged and His-tagged, are compatible. Biotinylated lipids are also compatible. Do not screen nanodiscs and detergents within the same cartridge unless advised by technical support.

Disclaimer

The compatibility table provides guidance on the impact of commonly used molecular biology compounds in CFPS reactions when preparing custom Cell-free Blends. While it helps reduce the risk of incompatibility, it does not cover all potential compounds or combinations of compounds. If you have concerns or need further insights with the use of your additives, please contact Technical Support before experimentation.

The listed additives have been tested for compatibility with expression, purification, and droplet movement on the eProtein Discovery system within listed tolerated additive concentration thresholds. Please note that Nuclera is not liable for the quality of chemicals prepared or for failed runs resulting from the improper use of these chemicals.

For use of nucleic acids (DNA/RNA) and/or protein additives, please contact Technical Support for more guidance.

Additives listed have been tested individually. When considering using custom additives, chemical cross-reactivity of combinations should be evaluated. The compatibility and performance of combinations of two or more additives have not been evaluated by Nuclera.

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Nuclera Technical Support:
UK Phone / WhatsApp Business: +44 1223 942 761
US Phone: +1 508-306-1297
Email: techsupport@nuclera.com

Offices:
Nuclera UK (HQ): One Vision Park, Station Road, Cambridge, CB24 9NP, UK
Nuclera USA: 1000 Technology Park Drive, Suite B, Billerica MA 01821, USA

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