VitroEase™ Buffer Screening Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ VitroEase™ Buffer Screening Kit enables efficient optimization of cryo-EM samples for single particle analysis. Sample screening evaluates protein behavior in various buffers with and without additives with the end goal of identifying the optimal conditions where the protein is structurally intact, randomly oriented, and equally distributed in ice. The kit contains a broad range of pre-formulated buffers and detergents (10X concentrations) that have been provided with color-coded caps that correlate with the color-coded screening strategy. The comprehensive sample screening strategy uses two rounds of screening and 24 conditions that can be screened in two working days on the microscope with the Autoloader. A condensed strategy is also provided using 8 conditions and taking one working day to screen in the microscope with the Autoloader.

Contents and storage

Product	Cat. No.	Kit Contents (Sufficient for up to 400 grids preparation)	Volume	Storage
VitroEase™ Buffer Screening Kit	A49856	**Buffer 1 (10X)**: $C_2H_3NaO_2$ (0.5 M), NaCl (1.5 M); pH 3.6	400 µL	Store at –20°C. Thaw reagents on ice before use. Product is stable through multiple freeze/thaw cycles.
		Buffer 2 (10X): $C_2H_3NaO_2$ (0.5 M), KCl (3 M); pH 3.6	400 µL	
		Buffer 3 (10X): MES (0.5 M), NaCl (1.5 M); pH 5.5	400 µL	
		Buffer 4 (10X): MES (0.5 M), KCl (3 M); pH 5.5	400 µL	
		Buffer 5 (10X): Tris-HCl (0.5 M), Mg $(CH_2COO)_2$ (0.1 M), NaCl (1.5 M); pH 7.2	400 µL	
		Buffer 6 (10X): Tris-HCl (0.5 M), MgCl2 (0.1 M), CH_3CO_2K (1.5 M); pH 7.2	400 µL	
		Buffer 7 (10X): Tris-HCl (0.5 M), $Mg(CH_3COO)_2$ (0.1 M), KCl (3 M); pH 7.2	400 µL	
		Buffer 8 (10X): HEPES (0.5 M), NaCl (1.5 M); pH 7.4	400 µL	
		Buffer 9 (10X): HEPES (0.5 M), KCl (3 M); pH 7.4	400 µL	
		Buffer 10 (10X): HEPES (0.5 M), $Mg(CH_3COO)_2$ (50 mM), CH_3CO_2K (1.5 M); pH 7.4	400 µL	
		Buffer 11 (10X): HEPES (0.5 M), $MgCl_2$ (50 mM), $CaCl_2$ (50 mM), NaCl (1.5 M); pH 7.4	400 µL	
		Buffer 12 (10X): PBS (1.37 M NaCl, 270 mM KCl, 43 mM Na_2HPO); pH 7.4	400 µL	
		Buffer 13 (10X): Bicine buffer (0.5 M), NaCl (1.5 M); pH 8.5	400 µL	
		Buffer 14 (10X): CAPSO (0.5 M), KCl (3 M); pH 8.9	400 µL	
		Detergent A (10X): CTAB (0.3%)	100 µL	
		Detergent B (10X): CHAPS (4.9%)	100 µL	
		Detergent C (10X): β-OG (2.7%)	100 µL	
		Detergent D (10X): Tween™ 20 (0.1%)	100 µL	
		Detergent E (10X): DM (1%)	100 µL	
		Detergent F (10X): FOM (0.7%)	100 µL	

Procedure summary

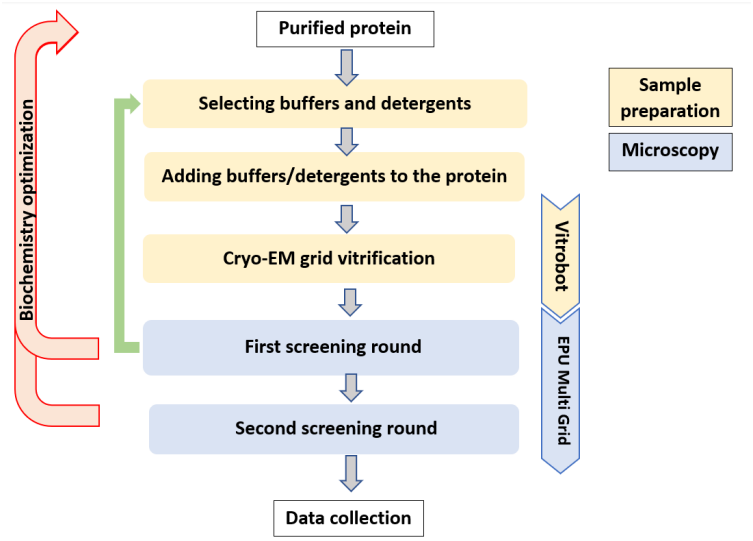

Figure 1 Workflow of sample optimization using the VitroEase™ Buffer Screening Kit.

Materials required but not supplied

- Protein sample (concentration ~1–10 mg/mL)
- P10 adjustable pipettes and tips
- Sample preparation reaction tubes (Cat. No. 4358297)
- *(Optional)* Mini desalting column (Cat. No. 89878)
- *(Optional)* Protein concentrator (Cat. No. 88535)
- Vitrobot Mark IV filter paper
- Vitrification tools (e.g., Vitrobot™ Mark IV System) (**http://www.thermofisher.com/nl/en/home/electron-microscopy/products/sample-preparation-equipment-em/vitrobot-system.html**)
- EM grid tweezer
- Cryo-EM grids (e.g., Quantifoil™ R 1.2/1.3 on 200 or 300 mesh)
- Auto grids and C-clip rings

Additional information

- Optimize vitrification settings independent of sample optimization.
- Use of detergents may affect protein distribution as well as the number of particles per hole.
- Sample optimization conditions are strongly dependent on the protein.
- Remove sugar, glycerol, and high salt concentration (>500 mM) from protein solution prior to using the optimization kit.
- Applied sample volume for vitrification is recommended for use with the Vitrobot™ Mark IV System. Different vitrification instruments may require different volumes and/or concentrations.
- The recommended screening strategy is based on using a Thermo Fisher Scientific Autoloader-based TEM-microscope: Krios™, Glacios™, or Talos™ Arctica™.
- Detergents and different pH can affect the structure and function of protein of interest. It is recommended that once an optimal condition for cryo-EM is found, a biochemical assay is used to verify the protein function.
- Grid type selection for cryo-EM sample preparation has to be verified prior to using screening strategy. It is not recommended to change grid type for different conditions during buffer optimization.
- Adding 10X buffers and detergents will dilute the protein samples, but the small volumes used should not significantly affect the cryo-EM applications .

Recommended comprehensive screening strategy

Table 1 Recommended comprehensive screening strategy.

First Screening Round		Second Screening Round	
1	Protein with lower concentration (1 mg/mL) in the purification buffer	1	Buffer (best condition from Round 1) + 0.5 µL × CMC CTAB
2	Protein with higher concentration (up to 10 mg/mL) in the purification buffer	2	Buffer (best condition from Round 1) + 0.75 µL × CMC CTAB
3	Buffer 1 or Buffer 2, pH 3.6	3	Buffer (best condition from Round 1) + 0.5 µL × CMC CHAPS
4	Buffer 3 or Buffer 4, pH 5.5	4	Buffer (best condition from Round 1) + 0.75 µL × CMC CHAPS
5	Buffer 6, pH 7.2	5	Buffer (best condition from Round 1) + 0.25 µL × CMC FOM
6	Buffer 7, pH 7.2	6	Buffer (best condition from Round 1) + 0.5 µL × CMC FOM
7	Buffer 10 or Buffer 11, pH 7.4	7	Buffer (best condition from Round 1) + 0.25 µL × CMC Tween-20™
8	Buffer 13, pH 8.5	8	Buffer (best condition from Round 1) + 0.5 µL × CMC Tween-20™
9	Buffer 14, pH 8.9	9	Buffer (best condition from Round 1) + 0.25 µL × CMC β-OG
10	Buffer 5, pH 7.2 + 0.1 µL × CMC β-OG or 0.75 µL × CMC DM	10	Buffer (best condition from Round 1) + 0.75 µL × CMC β-OG
11	Buffer 8, pH 7.4 + 0.75 µL × CMC Tween-20™ or 0.75 µL × CMC FOM	11	Buffer (best condition from Round 1) + 0.25 µL × CMC DM
12	Buffer 9, pH 7.4 + 0.1 µL × CMC CHAPS or 0.1 µL × CMC CTAB	12	Buffer (best condition from Round 1) + 0.5 µL × CMC DM

Round 1: Protein concentration optimization (2 grid conditions)

Note: Higher protein concentrations may improve the protein stability and distribution on the cryo-EM grid. Starting protein concentration for optimization of cryo-EM sample preparation is ~3–10 times higher than the concentration used for a negative stain grid.

1. Prepare one grid with lower protein concentration (e.g., 0.5–2 mg/mL) to separate particles for better recognition of the particles.
2. Prepare a second grid at the highest available protein concentration up to 10 mg/mL to identify whether the protein is amenable to close packing (sometimes referred to as carpets or monolayers).
3. Mix the freshly made solution well and apply 3 µL of mixture to a freshly glow discharged EM grid and start the plunge freezing process.

Round 1: Buffer and pH selection (7 grid conditions)

Kit buffers are provided with pH ranges between 3.6 and 8.9, pre-mixed with various salts and divalent cations. The conditions are color-coded in the table and match the tube cap colors for ease-of-use.

Use Buffers 1-14 based on choices using the Screening table.

1. Prepare one grid with the buffer condition that is most similar to the original purification buffer.
2. Prepare additional six grids to test ideal pH and salt concentrations as shown in first screening round. Buffers have been provided at pH values with two salt conditions. There are also buffers 6, 10 and 11 in neutral pH to test protein behavior in ice with addition of cationic ions.
3. Thoroughly mix the freshly made solution, apply 3 µL of mixture to a freshly glow-discharged EM grid, and start the plunge-freezing process.

   Example: Select one condition with a low pH value such as Buffer 3 (50 mM MES, 150 mM NaCl, pH 5.5). Select the buffer with the same pH with varying salt concentrations to test the requirements for ionic strength and protein stability such as Buffer 4, (50 mM MES, 300 mM KCl, pH 5.5).

Mix buffers and additives just before vitrification. Do not incubate the solution for excessive time. Do not mix different buffers from the kit for one sample preparation.

Round 1: Detergent screening (3 grids)

The purpose of using detergent in the first screening round is to determine the effect detergent charge, concentration, and CMC on protein behavior in ice.

1. Prepare mixture of Protein Solution with 10X Buffer and Detergent as indicated in screening strategy Round 1.
2. Thoroughly mix the freshly made solution, apply 3 µL of mixture to a freshly glow-discharged EM grid, and start the plunge-freezing process.

Possible outcomes with detergent screening in Round 1

- If there was no improvement in particle orientation after adding detergents, the concentration of detergent should be increased up to its CMC value in the second screening round.
- If the number of particles decreased significantly after adding a detergent, the protein concentration needs to be increased.
- If there was visible improvement, then the first screening result will direct the user to determine the buffer and detergent selection for the second screening round.
- If there was no visible improvement in the first screening round, then the sample stability and integrity should be improved in the upstream protein purification.

Round 2: Detergent optimization (12 grids)

Note: Use the optimal Buffer and Protein Concentration determined in the Round 1 screening.

1. Prepare the mixture of Protein Solution with 10X Buffer, as indicated in the screening strategy for Round 2.
2. Thoroughly mix the freshly made solution, apply 3 µL of mixture to a freshly glow-discharged EM grid, and start the plunge-freezing process.

   Note: If the number of particles decreased after adding a detergent, the protein concentration needs to be increased.

Limited screening round (8 grids)

The table below provides guidelines for screening a limited number of conditions for protein optimization on a grid.

Note: The first 4 conditions can be prepared in duplicate.

Table 2 Limited screening strategy.

Limited Screening Strategy		
1	Low pH buffer Buffer 3, pH 5.5	1 µL Buffer 3 + 9 µL protein solution
2	Neutral pH, High salt Buffer 7, pH 7.2	1 µL Buffer 7 + 9 µL protein solution
3	Neutral pH 7.4 buffer + 0.5 µL× CMC CTAB	1 µL Buffer 11 + 9 µL protein solution
4	Neutral pH 7.4 buffer + 0.5 µL × CMC CHAPS	1 µL neutral (pH 7.4) buffer + 9 µL protein solution + 0.5 µL CHAPS
5	Neutral pH + Cationic ions Buffer 11, pH 7.2	1 µL neutral (pH 7.4) buffer + 9 µL protein solution + 0.5 µL CTAB
6	Neutral pH 7.4 + 0.5 µL × CMC β-OG	1 µL neutral (pH 7.4) buffer + 9 µL protein solution + 0.5 µL β-OG
7	Neutral pH 7.4 + 0.5 µL × CMC FOM	1 µL neutral (pH 7.4) buffer + 9 µL protein solution + 0.5 µL FOM
8	Neutral pH 7.4 + 0.5 µL × CMC DM	1 µL neutral (pH 7.4) buffer + 9 µL protein solution + 0.5 µL DM

Recommended conditions in limited screening round

- Prepare mixture of Protein Solution (determined in protein concentration optimization), 10X Buffer, and Detergent indicated in the table above.
- Thoroughly mix the freshly made solution, apply 3 µL of mixture to a freshly glow-discharged EM grid, and start the plunge-freezing process.

Note: If the number of particles decreased after adding a detergent, the protein concentration needs to be increased.

Table 3 Detergent CMC guidelines for additional optimization.

Detergents	Class/Head Group	CMC	Stock Concentration	0.1 × CMC	0.25 × CMC	0.5 × CMC	0.75 × CMC
CTAB (Hexadecyl-trimethyl-ammonium bromide	Cationic	0.03%	0.3%	0.1 µL	0.25 µL	0.5 µL	0.75 µL
CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate)	Zwitterionic	0.49%	5%	0.15 µL	0.24 µL	0.49 µL	0.74 µL
β-OG (Octyl-β-D-Maltoside)	Non-ionic	0.27%	2.7%	0.1 µL	0.25 µL	0.5 µL	0.75 µL
Tween-20™ (Polysorbate 20)	Non-ionic	0.01%	0.1%	0.1 µL	0.25 µL	0.5 µL	0.75 µL
DM (n-Decyl-β-D-Maltoside)	Non-ionic	0.09%	1%	0.1 µL	0.25 µL	0.5 µL	0.75 µL
FOM (Fluorinated Octyl Maltoside)	Non-ionic	0.07%	0.7%	0.1 µL	0.25 µL	0.5 µL	0.75 µL

Troubleshooting

Observation	Possible cause	Recommended action
Detergent or buffer solution volume, color, or viscosity changed.	Wrong product storage condition was used.	Store at recommended condition.
	Precipitate formed due to slow freeze/thaw cycle.	Heat back into solution for 5–10 minutes in a 37°C water bath.
		Perform a quick freeze/thaw cycle for 5 minutes in a 37°C water bath.
Prepared cryo-EM sample is not easy to pipet for loading on the EM grid.	High viscosity formed due to wrong dilution factor being used for the cryo-EM sample preparation.	Make a 1:10 dilution of Kit buffer (10X):Protein solution.
Vitreous ice is too thick or thin.	Non-optimized vitrification settings were used.	Adjust blotting time and force.
Sample is falling apart in vitreous ice.	Unsuitable vitrification buffer was used.	Test various buffers with different pH, salt, and detergent.
	Upstream sample optimization was required.	Optimize protein purification steps and verify protein stability by biochemical assays prior to vitrification.
Not all protein orientations are present.	Particle absorbance occurred at the air to water interface. (Compromised by the tendency of proteins to align in non-random ways.)	Use various buffers with different pH, salt, and additive.
		Use the recommended detergent screening in the second screening round.
Low image contrast/low signal-to-noise ratio.	Incompatible compound was used in the cryo-EM procedure.	Remove a compound from the protein solution (e.g., by using Zeba™ columns or dialysis membranes).
Decrease in particle number after detergent addition.	Suboptimal protein to detergent concentration was used.	Increase protein concentration in the presence of detergent.
		Decrease the detergent concentration.

Limited product warranty

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Revision	Date	Description
A.0	16 April 2021	New document.