

User Manual PrecisION[®] Ion Channel Cell Lines

Validated Cell Lines for Use in Cell-Based Functional Assays Including Manual and Automated Patch Clamp or Fluorescent Platforms





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Overview

PrecisION cell lines are designed to provide pharmacologically relevant cell models for research and screening applications.

PrecisION Cell Lines

PrecisION cell lines stably express the target of interest in cell backgrounds that are highly suitable for electrophysiology applications.

Materials Provided

| Components | |
|------------------|--|
| 2 vials of cells | Refer to cell line-specific datasheet for shipped cell density |

Storage Conditions

Cells are shipped on dry ice and should arrive in a frozen state. To ensure maximum cell viability, store the vials of cells in the vapor phase of liquid nitrogen as soon as possible upon receipt. Please contact technical support immediately, if the cells received were already thawed.



Contact technical support immediately if cells received were already thawed.

- Short-term (less than 24 hours): Store vials at -80°C immediately upon arrival. (DO NOT store at -80°C for longer than 24 hours).
- Long-term (longer than 24 hours): Vials should ONLY be stored in liquid nitrogen (vapor phase ONLY) or in ultra-low temperature (-150°C) freezers.

Additional Materials Required

Refer to the table below to determine the appropriate media and reagents required for the specific cell line used in the assay. The cell type is provided in the cell line-specific datasheet.

| Material | Ordering Information Recommended Products (or Equivalent) | | | |
|--|---|--|--|--|
| Cell line-specific Complete Media | Refer to the tables in Appendix 1 for cell line-specific media requirements | | | |
| PBS, pH 7.4 (without calcium and magnesium) | ThermoFisher 10010-031 (or equivalent) | | | |
| AssayComplete [™] Cell Detachment Reagent | Eurofins DiscoverX Cat # 92-0009 | | | |
| Single and multichannel micropipettes and pipette tips | 3 | | | |
| 50 mL and 15 mL polypropylene tubes | | | | |
| 1.5 mL microtubes | | | | |
| Tissue culture disposable pipettes (1 mL-25 mL) and t | tissue culture flasks (T25, T75 and T225 flasks, etc.) | | | |
| Cryovials for freezing cells | | | | |
| Automated Cell Counter (or Hemocytometer) | | | | |
| Humidified tissue culture incubator (37°C and 5% CO | 2) | | | |

Unpacking Cell Cryovials

Two cryovials are shipped on dry ice, and each contain cells in 1 mL of Freezing Reagent (refer to the cell linespecific datasheet for the number of cells provided in the vials). The following procedures are for safe storage, handling and removal of cryovials from the vapor phase of liquid nitrogen storage.

1. Cells must arrive in a frozen state on dry ice.



Contact technical support immediately if cells received were already thawed.

 Frozen cells must be transferred to either the vapor phase of liquid nitrogen or a -80°C freezer immediately upon arrival. If cells will be thawed and used within 24 hours, they can be stored temporarily at -80°C. For long-term storage, cryovials must be stored in the vapor-phase of liquid nitrogen.



Cryovials should be stored in the vapor phase of liquid nitrogen. They are not rated for storage in the liquid phase.

3. Using tongs, remove the cryovials from the liquid nitrogen storage, and place them immediately in dry ice, in a covered container.



A face shield, gloves, and a lab coat should be worn during these procedures.

- 4. Wait for at least one minute for any liquid nitrogen that may be present inside the vial to evaporate.
- 5. Proceed with the thawing and propagation protocol in the following section. Refer to the cell line-specific datasheet for appropriate products mentioned in the following protocols.

Cell Culture Protocol

The following procedures are for thawing adherent cells from cryovials, seeding and expanding the cells, and freezing the cells once they have been propagated. Refer to the cell-line specific datasheet to identify the cell type of the cell line. Refer to Appendix 1 to determine the correct complete media formulation for the specific cell type.

Cell Thawing_

The following is a protocol for thawing cells into a T75* flask. (*T25 flask where noted)

- 1. Pre-warm the complete media in a 37°C water bath for 15 minutes, as indicated on the cell line-specific datasheet.
- Add 9 mL of the complete media into a 15 mL conical tube inside a sterile tissue culture hood. Set aside for Step 6.
- 3. Remove the cryovials from -80°C or liquid nitrogen vapor storage and immediately place them in dry ice prior to thawing.



Safety Warning: A face shield, gloves, and a lab coat should be worn at all times when handling frozen vials. When removing cryovials from the vapor phase of liquid nitrogen storage, use tongs to place them immediately in dry ice in a covered container. Wait at least 1 minute for any liquid nitrogen inside the vial to evaporate. Do not touch the bottom of the vials at any time, to avoid inadvertent thawing of the cells.

 Place the cryovials containing the cells in a 37°C water bath briefly, until only small ice crystals remain and the cell pellet is almost completely thawed. The thawing time typically ranges from 1.5 to 2.5 minutes. DO NOT vortex freshly thawed cells.



Do not leave the frozen cell vials unattended in the water bath. Prolonged thawing at 37°C may result in cell death.

- 5. Decontaminate the external surface of the vials by spraying and wiping them with 70% ethanol. Transfer the vials to a tissue culture hood.
- 6. Using a sterile pipette, gently transfer the thawed cells to the pre-filled 15 mL conical tube.
- 7. Rinse the vial with 1 mL of complete media to maximize cell recovery and pipette it into the 15 mL conical tube.
- 8. Centrifuge the 15 mL conical tube containing cells at 250 x g for 4 minutes.
- 9. Add 18 mL of complete media to a T75 flask (or 6 mL to a T25 flask for the cell lines noted below). Some cell lines recover better when thawed into a T25 flask (CYL3054, CYL3056, and CYL3096).
- 10. After centrifugation, aspirate the media from the conical tube with care not to disturb the cell pellet.
- 11. Gently resuspend the pellet with 2 mL of complete media.
- 12. Transfer the cell suspension to the T75 (or T25 where noted) flask and gently swirl the flask to distribute the cells evenly in solution.
- 13. Incubate the flask in a 37°C and 5% CO₂ humidified cell culture incubator.
- 14. Maintain the cells in culture until they are 60-75% confluent in the T75 (or T25 flask). Do not exceed 80% confluency, as target down-regulation may occur.
- 15. When passaging cells, refer to the instructions in the Cell Recovery section.

16. We recommend maintaining the cultures in T225 flasks, being careful not to exceed 80% confluency. All seeding densities in this manual are those recommended for T225 flasks. If other sized flasks are used, adjust seeding densities appropriately, according to the surface area of the flask.

Cell Recovery_____

The following is a protocol for ensuring maximal cell recovery once the cells become 60-80% confluent in a T225 flask.

- 1. Pre-warm the complete media in a 37°C water bath for 15 minutes.
- 2. Remove the T225 flask from the tissue culture incubator and place it in a sterile tissue culture hood.
- 3. Gently aspirate the media from the T225 flask.
- 4. Add 15 mL of PBS into the T225 flask, and gently rock the flask back and forth to rinse the cells.
- 5. Gently aspirate PBS from the flask.
- 6. Add 4 mL of pre-warmed Cell Detachment Reagent to the flask. Gently rock the flask back and forth to ensure that the inner surface of the flask is uniformly covered with Cell Detachment Reagent.
- Incubate the flask at 37°C and 5% CO₂ until the cells have detached. If the cell line is a HEK 293 background, the maximum amount of time for Cell Detachment Reagent to be on the cells is about 2 minutes. If the cell line is a CHO-K1 background, the maximum amount of time is about 3 minutes.



The maximum amount of time for Cell Detachment Reagent to be on the cells is different depending on the background. Do not leave Cell Detachment Reagent on cells longer than necessary.

 Remove the flask from the incubator and observe the cells under a microscope to confirm cell detachment. If necessary, gently tap the edge of the flask to detach cells from the surface. If most of the cells have not yet detached, then return the flask to the incubator for additional 1 minute. Repeat this step until all cells are in suspension.



Some cell lines are highly adherent and therefore may require additional dissociation time.

- 9. Add 10 mL of pre-warmed complete media to a 50 ml centrifuge tube.
- 10. Using a pipette, slowly add 16 mL of the complete media to the detached cells in T225 flask to gently rinse them into the suspension. Gently pipet the cell suspension up and down several times to generate a single cell suspension without any clumps.
- 11. Count the number of viable cells using an automated cell counter or hemocytometer. Determine the number of cells and cell suspension volume required to seed into a T225 flask. Cells should be initially seeded according to Appendix 2. Please Note: These are initial seeding recommendations for the first passages out of thaw. Seeding densities should be adjusted based on the desired time window for expression, generally recommended to be 48 72 hours. Cell growth rates will vary depending on the amount of time in continuous culture, and the density of cells prior to lifting them during recent passages.
- 12. Add 15 mL of growth media to a new T225 flask, followed by addition of the cell suspension. Add an additional volume of the growth media to reach a final volume of 54 mL for a T225flask.
- 13. Transfer the flask to a tissue culture incubator and incubate the cells at 37°C and 5% CO₂.

Cell Propagation

The cells can be propagated after a successful recovery, which can be determined by monitoring them under a microscope. Healthy cells should adhere uniformly to the surface of the flask, with only a few cells remaining in suspension.



To maintain logarithmic growth of the cells, cultures should be maintained in a sub-confluent monolayer.

- 1. If the cells do not appear to be healthy, or if confluency is <25% (Ion Channel cell lines may demonstrate unusual morphology at low densities and within 72 hours of thawing):
 - 1.1. Incubate the flask for additional 24 to 48 hours to allow for cell recovery before exchanging the cell culture media. If culture has been static for more than 3 days, a media exchange may be required. Some lines may require up to 5 days to recover from being thawed.
- 2. Once the cells have reached 65-75% confluency, split the cells every 2 to 3 days, based on the doubling time of the cell line. Refer to Appendix 2 for cell seeding recommendations.

Cell Cryopreservation_____

The following procedure is for freezing cells that have been propagated in T225 flasks. This protocol assumes that the cells have reached 65-75% confluency in a sufficient number of flasks to fill the required number of cryovials. The cells will be resuspended in ice-cold CryoMedium (described in Step 15), and will be cryopreserved in aliquots of 1 mL/vial, at the desired number of cells per vial (e.g. 2.0 x 10⁶ per vial). Refer to the table below for the ratio of components used to prepare the CryoMedium:

| Basal Media (Without Selection Antibiotics) | 40% |
|--|-----|
| Fetal Bovine Serum (FBS) | 50% |
| Cell-culture grade Dimethyl Sulfoxide (DMSO) | 10% |

1. Remove T225 flasks from the incubator and place them in a sterile tissue culture hood.



Care should be taken while handling flasks to avoid contamination.

- 2. Slowly aspirate the media from the flasks.
- Add 15 mL of PBS into each T225 flask, and gently rock the flask back and forth to ensure that the cells are rinsed.
- 4. Gently aspirate PBS from the flask.
- 5. Add 4 mL of pre-warmed Cell Detachment Reagent to T225 flask.
- 6. Gently rock the flask back and forth to ensure that the inner surface of the flask is uniformly covered with Cell Detachment Reagent.
- Incubate the flask at 37°C and 5% CO₂ until the cells have detached. If the cell line is a HEK 293 background, the maximum amount of time for Cell Detachment Reagent to be on the cells is 2 minutes. If the cell line is a CHO-K1 background, the maximum amount of time is 3 minutes.



ne maximum amount of time for Cell Detachment Reagent to be on the cells is different depending on the background. Do not ave Cell Detachment Reagent on cells longer than necessary. Doing so may disrupt the ion channel function.

8. Remove the flask from the incubator and view under a microscope to confirm that the cells have detached. If necessary, gently tap the edge of the flask to detach cells from the surface. If most of the cells have not

detached, then return the flask to the incubator for additional 1 minute and repeat this step until all cells are in suspension.

- 9. Add 16 mL of the cell culture media to each T225 flask to neutralize the Cell Detachment Reagent
- 10. Gently pipet the cell suspension up and down several times to generate a single cell suspension without any clumps.
- 11. Transfer the cell suspension from the T225 flask into a 50 mL conical centrifuge tube. If necessary, add an additional volume of the cell culture media to the flasks and rinse to collect the remaining cells, then transfer it into the conical tube. Slowly pipette up and down several times to ensure that a single cell suspension is formed.
- 12. To determine the number of cells in the suspension:
 - 12.1. Set aside a small fraction (0.5 mL or less) of the suspended cells in a separate tube.
 - 12.2. Transfer an appropriate portion of this fraction to a cell counter or hemocytometer.
 - 12.3. Count cells, calculate the concentration of cells in the suspension, and then calculate the total number of cells in the 50 mL centrifuge tube.
- 13. Centrifuge the collected cells at 200 x g for 4 minutes.
- 14. After centrifugation, discard the supernatant, being careful not to disturb the cell pellet.
- 15. Based on the total cell number calculated in Step 12, resuspend the cells to the desired concentration (e.g. 2.0 x 10⁶ cells/mL) with ice-cold CryoMedium.



Keep cells on ice during this process to protect cell viability.

- 16. Aliquot 1 mL of the cell suspension into each of the labeled 2 mL cryovials. Seal the cryovials tightly.
- 17. Freeze cells in a -80°C freezer at a controlled rate of -1°C/minute overnight in a dedicated cell freezer or commercially-available freezing chamber. For short-term storage, vials can be stored in the -80°C freezer for a maximum of two weeks.
- 18. Transfer the vials into the vapor phase of liquid nitrogen for long-term storage.

Recommendations for Testing

General guidelines are provided for cell seeding but may vary based on recording conditions and equipment used for assay. For running the assay, refer to relevant protocols as recommended by the equipment manufacturer or published literature.

1. Preparation of Cells_____

The following protocol provides steps for harvesting and preparing adherent cells. This protocol assumes that cells have reached a 65-75% confluency and have been cultured in the recommended cell culture media. For cell line-specific information on the control compound, incubation times and temperature schedule, refer to the cell line-specific datasheet.

- 1.1. Ensure that the cells are in the logarithmic growth phase prior to seeding for use in the assay.
- 1.2. Warm the cell culture media in a clean 37°C water bath for 15 minutes.
- 1.3. Gently harvest the cells with the method-specific dissociation and assay preparation protocol.

2. Compound Preparation

For compounds prepared in DMSO, ensure final DMSO concentration in assay is < 0.3 - 0.5%.

Appendix 1: Complete Media Formulations – Parental Cell Lines Growth media used for the parental cell lines are listed in the following table.

| Complete Media | Target | Catalog Number | Component | Supplier Name | Supplier Part Number |
|-------------------|----------------|-------------------|---------------------------------|--------------------|----------------------------|
| HEK Wild | HEK 293 WT1 | CYL3001 | DMEM/F-12 (with LGlutamine) | ThermoFisher | 10565-018 |
| Type Media | HEK 293 WT2 | CYL3002 | 10% FBS | Millipore Sigma | F2442 |
| | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| CHO Wild | CHO-K1 WT | CYL3000 | F-12 (Ham's) + LGlutamine | ThermoFisher | 3176-035 |
| Type Media | | | 10% FBS | Millipore Sigma | F2442 |

Appendix 1: Complete Media Formulations – Recombinant Cell Lines Growth media used for each cell line background are listed in the following tables.

| Complete Media | Target | Catalog Number | Component | Supplier Name | Supplier Part Number |
|-------------------|--------------|-------------------|---------------------------------|--------------------|----------------------------|
| HEK Media 1 | hNav1.5 | CYL3004 | DMEM/F-12 (with L-Glutamine) | ThermoFisher | 10565-018 |
| | hNav1.1 | CYL3009 | | | E0 () () |
| | hNav1.6 | CYL3010 | 10% FBS | Millipore Sigma | F2442 |
| | hNav1.7 | CYL3011 | 1% Non-Essential | ThermoFisher | 11140-050 |
| | hNav1.4 | CYL3024 | Amino Acids | | |
| | hKir2.1 | CYL3032 | 400 µg/mL G418 | Eurofins | 92-0030 |
| | hERG- HEK | CYL3039 | | DiscoverX | |
| | hHCN1 | CYL3040 | | | |
| | hHCN2 | CYL3041 | | | |
| | hHCN3 | CYL3042 | | | |
| | hGluR6 | CYL3049 | | | |
| | hASIC3 | CYL3055 | | | |
| | hTRPV1 | CYL3063 | | | |
| | hTRPV4 | CYL3064 | | | |
| | hTRPV3 | CYL3065 | | | |
| | hTRPA1 | CYL3066 | | | |
| | hCav3.2 | CYL3075 | | | |
| | hCFTR | CYL3088 | | | |
| | hKv12.2 | CYL3089 | | | |

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|---------------------------------------|----------------------------|--|---------------------------------|-----------------------|-----------|
| HEK Media 2 | hNav1.8/ β1 | CYL3025 CYL3053 | DMEM/F-12 (with LGlutamine) | ThermoFisher | 10565-018 |
| | hGABAA α1/β3/γ2 | CYL3054 | 10% FBS | Millipore Sigma | F2442 |
| | hCav2.2 α1Β/β3/α 2δ1 | (Thaw into T25 flask) | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | hGABAA α3/β3/γ2 | CYL3068 | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 |
| | hGABAA α5/β3/γ2 | CYL3073 | 100 μg/mL Hygromycin | Eurofins DiscoverX | 92-0029 |
| | hGABAA α4/β3/γ2 | CYL3085 | 0.625 µg/mL Puromycin | Eurofins DiscoverX | 92-0028 |
| | hGABAA α6/β3/γ2 | CYL3086 | | | |
| | hnAChR α4/α6/β2 | CYL3107 | | | |
| HEK Media 3 | hKv7.4 | CYL3092 | DMEM/F-12 (w/ LGlutamine) | ThermoFisher | 10565-018 |
| | hKv7.4/K v7.5 | CYL3096 (Thaw into T25 flask) | 10% FBS | Millipore Sigma | F2442 |
| | hnAChR α4/β2 | CYL3106 | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 200 µg/mL Hygromycin | Eurofins DiscoverX | 92-0029 |
| HEK Media 4 | hnAChR α7/ric3 | CYL3097 | DMEM/F-12 (with L-Glutamine) | ThermoFisher | 10565-018 |
| | 21,1100 | | 10% FBS | Millipore Sigma | F2442 |
| | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 400 μg/mL G418 | Eurofins DiscoverX | 92-0030 |
| | | | 0.625 µg/mL Puromycin | Eurofins DiscoverX | 92-0028 |

| HEK Media 5 | nAChR α1/β1/δ/ε | CYL3052 | DMEM/F-12 (with L-Glutamine) | ThermoFisher | 10565-018 |
|----------------|-----------------------------|------------------------------|---------------------------------|-----------------------|-----------|
| | hnAChR α3/β4 | CYL3057 | 10% FBS | Millipore Sigma | F2442 |
| | hKv4.3/K ChiP2 | CYL3069 | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 400 μL/mL G418 | Eurofins DiscoverX | 92-0030 |
| | | | 100 µg/mL Hygromycin | Eurofins DiscoverX | 92-0029 |
| HEK Media 6 | hKv7.3/K v7.5 | CYL3060 | DMEM/F-12 (with L-Glutamine) | ThermoFisher | 10565-018 |
| | hKir6.2/S UR2A (KATP) | CYL3099 | 10% FBS | Millipore Sigma | F2442 |
| | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 |
| | | | 200 µg/mL Hygromycin | Eurofins DiscoverX | 92-0029 |
| HEK Media 7 | hGlyRA1 | CYL3056 (Thaw into T25 | DMEM/F-12 (with L-Glutamine) | ThermoFisher | 10565-018 |
| | | flask) | 10% FBS | Millipore Sigma | F2442 |
| | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 |
| | | | 30 µM Picrotin | Millipore Sigma | Y0001508 |

| HEK Media 8 | Cav1.2 α1C/β2a/ α2δ1 | CYL3051 | DMEM/F-12 (with L-Glutamine) | ThermoFisher | 10565-018 |
|----------------|----------------------------|--------------------|---------------------------------|-----------------------|-----------|
| | | | 10% FBS | Millipore Sigma | F2442 |
| | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 |
| | | | 100 µg/mL Hygromycin | Eurofins DiscoverX | 92-0029 |
| | | | 0.625 µg/mL Puromycin | Eurofins DiscoverX | 92-0028 |
| | | | 144 nM Nitrendipine | Millipore Sigma | N144 |
| CHO Media 1 | hNav1.3 hHCN4 | CYL3003 CYL3012 | IMDM (with L-Glutamine) | ThermoFisher | 12440-053 |
| | hNav1.2 | CYL3023 | 10% Dialyzed FBS | ThermoFisher | 26400-044 |
| | | | 1% HT Supplement | ThermoFisher | 11067-030 |
| | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 |

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|----------------|-----------------|---------|---------------------------------|-----------------------|-----------|
| CHO Media 2 | hKv1.1 | CYL3014 | F-12 (Ham's) + L-Glutamine | ThermoFisher | 31765-035 |
| | hKv1.2 | CYL3015 | | | |
| | hKv1.3 | CYL3016 | 10% FBS | Millipore Sigma | F2442 |
| | hKv1.4 | CYL3017 | 400 µg/mL G418 | Eurofins | 92-0030 |
| | hKv1.5 | CYL3018 | | DiscoverX | |
| | hKv1.6 | CYL3019 | | | |
| | hKv1.7 | CYL3020 | | | |
| | hKv1.8 | CYL3021 | | | |
| | hKv2.1 | CYL3022 | | | |
| | hERG- CHO | CYL3038 | | | |
| | hKv3.1 | CYL3043 | | | |
| | hKv3.2 | CYL3044 | | | |
| | hKv3.3 | CYL3045 | | | |
| | | | | | |
| CHO Media 3 | hKCNQ1 /minK | CYL3007 | IMDM (with L-Glutamine) | ThermoFisher | 12440-053 |
| | | | 10% Dialyzed FBS | ThermoFisher | 26400-044 |
| | | | 1% HT Supplement | ThermoFisher | 11067-030 |
| | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 |
| | | | 100 µg/mL Hygromycin | Eurofins DiscoverX | 92-0029 |

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|-----------------|-------------------|--------------------|--|---------------------------------|---------------------------------------|-----------|---------------------------------|--------------|-----------|
| CHO Media 4 | hKv4.2/K CHiP2 | CYL3026 | F-12 (Ham's) + L-Glutamine | ThermoFisher | 31765-035 | | | | |
| | hKv7.2/K v7.3 | CYL3059 CYL3067 | 10% FBS | Millipore Sigma | F2442 | | | | |
| | hKv2.1/K v9.2 | 0123007 | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 | | | | |
| | | | 100 µg/mL Hygromycin (Gibco) | Eurofins DiscoverX | 92-0029 | | | | |
| CHO Media 5 | hKv4.3/K CHiP1 | CYL3027 | DMEM (with L-Glutamine) | ThermoFisher | 11966-025 | | | | |
| | | | 10% FBS | Millipore Sigma | F2442 | | | | |
| | | | | | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 |
| | | | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 | | | | |
| ND7-23 Media | rNav1.8 | CYL3050 | DMEM (with L-Glutamine) | ThermoFisher | 11966-025 | | | | |
| | | | 10% Heat-Inactivated FBS** | Millipore Sigma | F2442 | | | | |
| | | | **Heat inactivation must be performed by user by incubating in a 60° water bath for at least 30 minutes. | | | | | | |
| | | | | 1% Non-Essential Amino Acids | ThermoFisher | 11140-050 | | | |
| | | | 400 µg/mL G418 | Eurofins DiscoverX | 92-0030 | | | | |

Appendix 2: Cell Line Specific Seeding Guidelines

Suggested seeding guidelines for each cell line background are listed in the following tables. Growth rate may vary due to amount of time in culture, and percent confluency at recent passages.

| Catalog Number | Cell Line | Seeding Density (x 10 ⁶) T225 flasks (48-72 hours) |
|----------------|-------------|--|
| CYL3000 | CHO-K1 WT | 6.0 |
| CYL3001 | HEK WT1 | 1.8 |
| CYL3002 | HEK WT2 | 1.8 |
| CYL3003 | hNav1.3 | 2.6 |
| CYL3004 | hNav1.5 | 5.3 |
| CYL3007 | hKCNQ1/minK | 1.8 |
| CYL3009 | hNav1.1 | 5.3 |
| CYL3010 | hNav1.6 | 5.3 |
| CYL3011 | hNav1.7 | . 5.3 |
| CYL3012 | hHCN4 | 2.0 |
| CYL3014 | hKv1.1 | 2.0 |
| CYL3015 | hKv1.2 | 2.0 |
| CYL3016 | hKv1.3 | 2.1 |
| CYL3017 | hKv1.4 | 2.1 |
| CYL3018 | hKv1.5 | 2.1 |
| CYL3019 | hKv1.6 | 2.1 |
| CYL3020 | hKv1.7 | 2.1 |
| CYL3021 | hKv1.8 | 2.1 |
| CYL3022 | hKv2.1 | 4.2 |

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|---------|------------------------|-----|
| CYL3023 | hNav1.2 | 2.6 |
| CYL3024 | hNav1.4 | 4.2 |
| CYL3025 | hNav1.8/β1 | 2.4 |
| CYL3026 | hKv4.2/KCHiP2 | 2.1 |
| CYL3027 | hKv4.3/KCHiP1 | 2.1 |
| CYL3032 | hKir2.1 | 2.1 |
| CYL3038 | hERG | 2.1 |
| CYL3039 | hERG | 5.3 |
| CYL3040 | hHCN1 | 8.3 |
| CYL3041 | hHCN2 | 8.3 |
| CYL3042 | hHCN3 | 2.0 |
| CYL3043 | hKv3.1 | 2.1 |
| CYL3044 | hKv3.2 | 2.1 |
| CYL3045 | hKv3.3 | 2.1 |
| CYL3049 | hGluR6 | 6.8 |
| CYL3050 | rNav1.8 | 2.4 |
| CYL3051 | Cav1.2 α1C/β2a/α2δ1 | 4.2 |
| CYL3052 | nAChR α1/β1/δ/ε | 2.4 |
| CYL3053 | hGABAA α1/β3/γ2 | 6.8 |
| CYL3054 | hCav2.2 α1Β/β3/α2δ1 | 6.0 |
| CYL3055 | hASIC3 | 2.4 |
| CYL3056 | hGlyRA1 | 6.8 |

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|---------|-------------------------|-----|
| CYL3057 | hnAChR α3/β4 | 6.0 |
| CYL3059 | hKv7.2/Kv7.3 | 2.1 |
| CYL3060 | hKv7.3/Kv7.5 | 2.1 |
| CYL3063 | hTRPV1 | 5.4 |
| CYL3064 | hTRPV4 | 2.4 |
| CYL3065 | hTRPV3 | 2.4 |
| CYL3066 | hTRPA1 | 2.4 |
| CYL3067 | hKv2.1/Kv9.2 | 2.1 |
| CYL3068 | hGABAA α3/β3/γ2 | 2.3 |
| CYL3069 | hKv4.3/KChiP2 | 2.1 |
| CYL3073 | hGABAA α5/β3/γ2 | 2.3 |
| CYL3075 | hCav3.2 | 1.8 |
| CYL3085 | hGABAA α4/β3/γ2 | 3.0 |
| CYL3086 | hGABAA α6/β3/γ2 | 4.5 |
| CYL3088 | hCFTR | 1.8 |
| CYL3089 | hKv12.2 | 2.3 |
| CYL3092 | hKv7.4 | 2.7 |
| CYL3096 | hKv7.4/Kv7.5 | 2.7 |
| CYL3097 | hnAChR α7/ric3 | 3.0 |
| CYL3099 | hKir6.2/SUR2A (KATP) | 3.0 |
| CYL3106 | hnAChR α4/β2 | 3.8 |
| CYL3107 | hnAChR α4/α6/β2 | 3.8 |
| | | |

Document Revision History

| Revision Number | Date Released | Revision Details |
|-----------------|---------------|------------------|
| 0 | March 2021 | New document |
| 1.0 | July 2023 | First Revision |
| 2.0 | October 2023 | Second Revision |

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