SGIDNA

# VMAX™ X2 CHEMICALLY COMPETENT CELLS

## Instructions

Catalog numbers CL1300-05, CL1300-10, CL1300-20

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# Product information

## Vmax™ X2 Chemically Competent Cells

| Component | Cat. no. CL1300-05 | Cat. no. CL1300-10 | Cat. no. CL1300-20 | Volume | Storage temperature |
|---|---|---|---|---|---|
| | Quantity | | | | |
| Vmax™ X2 Chemically Competent Cells | 5 vials | 10 vials | 20 vials | 50 µL/vial | −80°C |
| Vmax™ Chemicompetent Cell Recovery Medium | 1 bottle | 1 bottle | 2 bottles | 10 mL/bottle | Room temperature |
| Positive control pACYC/ Chlor plasmid | 1 vial | 1 vial | 1 vial | 25 µL at 5 ng/µL | −20°C |

## Accessory products

- Vmax™ Enriched Growth Medium (SGI-DNA cat. no. CL1500-1000)
- Vmax™ Chemicompetent Cell Recovery Medium (SGI-DNA cat. no. CL1520-10, CL1520-6X10)

## Guidance and recommendations

- Cells are shipped on dry ice. Upon receipt, store immediately at -80°C and protect cells from temperature fluctuations.
- Use competent cells within six months of receipt.
- Thaw Vmax™ X2 Chemically Competent Cells on ice just prior to use.
- If cells need to be resuspended, do so by gently flicking the tubes. Do not vortex or pipette up and down.
- Only use the provided Vmax™ Chemicompetent Cell Recovery Media for recovery after transformation. Do not use SOC or other recovery media.
- Pre-warm recovery media to 37°C prior to use.
- Store transformed Vmax™ X2 plates at room temperature for at least ten days, during which time colonies may be used for inoculation and/or re-streaking onto fresh plates.
- Store Vmax™ Enriched Growth Medium at room temperature and add antibiotics on an as-needed basis.
- Neither plates or liquid cultures of Vmax™ should be stored at 4°C.
- For long-term storage prepare glycerol stocks and store at -80°C.

# Overview

## Vmax X2 Chemically competent cells

Vmax™ X2 cells are an engineered *Vibrio natriegens* strain containing a major extracellular nuclease knockout and insertion of an IPTG-inducible T7 RNA polymerase cassette for expression of genes under a tightly controlled, inducible T7 promoter. A high transformation efficiency of over 1 x $10^7$ CFU/µg DNA, robust expression system, and extremely fast growth rate make Vmax™ X2 cells ideal for replacing traditional alternative strains in most protein expression workflows.

## Introduction

Vmax™ X2 is a novel bacterial strain and next-generation platform for recombinant protein expression. The Vmax™ X2 system is designed for fast growth and high protein yields using plasmids and workflows used for *E. coli*. Unlike other commonly used prokaryotic recombinant protein expression systems, Vmax™ X2 is derived from the marine microorganism, *Vibrio natriegens*[1,2]. This gram-negative, non-pathogenic bacterium exhibits the fastest growth rate of any known organism with a doubling time of less than 14 minutes, a growth rate that is twice as fast as that of *E. coli*[3].

The extremely fast growth rate of Vmax X2 provides important flexibility for demanding research environments, it responds well to a variety of nutrients and media, and it can outgrow *E. coli* and other protein expression systems at a range of temperatures (25°C to 37°C)[4,5]. This provides the opportunity to fine-tune protein expression rapidly under a variety of conditions at a smaller scale, then transition to large-scale production and purification.

To allow the seamless transfer of your *E. coli*-based expression constructs, Vmax™ X2 is compatible with a variety of commonly used protein expression vectors. This includes the pET-derived series of vectors that use the phage T7 expression system regulated by the addition of Isopropyl β-D-1-thiogalactopyranoside (IPTG). High transformation efficiencies also rival the best alternative bacterial expression strains with 1-5 x $10^7$ colonies formed per µg DNA, allowing for an easy switch to the Vmax workflow.

Similar to other historically used bacterial expression strains such as *E. coli* BL21(DE3), Vmax™ X2 can be cultured with routine growth medium such as Luria Broth (LB) supplemented with salt, 2xYT, Terrific broth (TB) and other commercial auto-inducible media. However, we recommend our animal component-free, optimized Vmax™ Enriched Growth Medium for best results.

Unlike *E. coli*, the induction of Vmax™ X2 is largely independent of growth phase and has been performed at a wide variety $OD_{600}$ of readings (0.1 to 1). Protein expression induced over this range remains stable with overnight induction and no negative impacts of expression have been observed. This flexibility in induction time gives you added convenience, reducing the need to closely monitor OD. Other advantages of Vmax™ X2 include:

- Typical protein yields 2- to 4-fold higher than *E. coli* due to Vmax's high biomass production and speed of growth.
- Efficient protein production with no restrictions on an optimal harvest point within a 4 to 24-hour period.
- A 100-fold reduction in endotoxin levels as compared to traditional *E. coli* strains.
- Figure 1 (on the following page) depicts the workflow comparison of *E. coli* and Vmax™ X2.

## The Vmax™ workflow can be completed in three days

| Standard *E. coli* workflow | | Vmax™ X2 workflow |
|---|---|---|
| 1. Transform competent cells<br>2. Recover<br>3. Plate on selective media<br>4. Grow overnight | **Day 1** ↓ | 1. Transform competent cells<br>2. Recover<br>3. Plate on selective media<br>4. Grow overnight |
| 5. Pick colony for seed culture<br>6. Grow overnight | **Day 2** ↓ | 5. Pick colony for starter culture<br>6. Grow 2 hours at 30°C<br>7. Split into two cultures, induce expression in one with IPTG<br>8. Grow 4–6 hours<br>9. Process culture for analysis and/or continue expression overnight |
| 7. Inoculate growth flask<br>8. Grow to OD600 of 0.5<br>9. Induce expression with IPTG<br>10. Express protein 4–6 hours<br>11. Pellet cells and freeze | **Day 3** ↓ | 10. Pellet cells<br>11. Perform analysis or protein purification |
| 12. Perform analysis or protein purification | **Day 4** | |

**Figure 1. Comparison of protein expression workflows**

## Table 1. Vmax™ X2 key features and advantages

| Key feature | Vmax™ X2 | *E. coli* | Vmax™ advantages over *E. coli* |
|---|---|---|---|
| **Doubling time** | < 14 minutes | ~30 minutes | • Fast growth rate<br>• Saves time |
| **Growth requirements** | Rapid growth from 25°C to 37°C | • Optimal at 37°C<br>• Slow growth below 37°C | • Potentially omit temperature-controlled shaker |
| **Biomass** | > 14 $OD_{600}$ | 8–10 $OD_{600}$ | • Faster and more cost-effective production of proteins<br>• Up to twice the biomass per volume of media |
| **Protein expression** | Produces more soluble protein with less optimization | Soluble protein expression is dependent on optimal induction OD and temperature | • More convenient<br>• Potential for higher rate of success with difficult-to-express proteins<br>• Higher soluble protein yields |

# Growing and maintaining transformed Vmax™ X2 cells

## Vmax™ growth media recommendations

A variety of media commonly used for *E. coli* are compatible with Vmax™ X2. However, Vmax™ X2 grows best in media with higher osmotic concentrations. Consequently, some commonly used media may require salt supplements. For optimal growth, we recommend Vmax™ Enriched Growth Medium (SGI-DNA Cat. no. CL1500-1000). This osmotically balanced, animal component-free, rich medium has been formulated specifically for Vmax™ to support fast doubling times and high-level protein expression.

### Table 2. Vmax™ X2 growth conditions in different media

| Media | Format | 25°C | 30°C | 37°C | Recommendation* |
|---|---|---|---|---|---|
| **Vmax™ Enriched Growth Medium (animal-free)** | Liquid culture | Growth | Growth | Growth | ++++ (Preferred) |
| **Enhanced 2xYT medium** | Liquid culture | Growth | Growth | Growth | +++ (Recommended) |
| **Brain heart infusion broth + v2 salts‡** | Liquid culture | Growth | Growth | Growth | +++ (Recommended) |
| **LB-Miller†** | Agar plate | Slow growth | Growth | Growth | +++ (Recommended) |
| **Brain heart infusion agar + v2 salts‡** | Agar plate | Growth | Growth | Growth | ++ |
| **MagicMedia™ *E. coli* Expression Medium** | Liquid culture | Not tested | Growth | Growth | ++ |
| **LB-Miller†** | Liquid culture | Growth | Growth | Supplement with v2 salt‡ | + |
| **Terrific broth** | Liquid culture | Not tested | Growth | Growth | + |

*Recommendation key:
++++ = Preferred media, +++ = Recommended, ++ = Acceptable, + = Acceptable, but other media may support better growth.
†LB-Miller medium contains 10 g/L of NaCl. Other LB variants (e.g. LB-Lennox and LB-Luria) contain less salt and will not support optimal growth.
‡v2 salt: 204 mM NaCl, 4.2 mM KCl, and 23.14 mM MgCl2 (these are in addition to salts present in base media).

## Antibiotic selection using Vmax™ X2

*E. coli* expression vectors and their corresponding antibiotic selection markers are compatible with Vmax™ X2. However, plasmid copy number and the minimum inhibitory concentration (MIC) may be different between Vmax™ and *E. coli*. Recommended concentrations for maintaining antibiotic selection using Vmax™ X2 culture on solid or liquid culture are listed in the following table.

### Table 3. Maintaining antibiotic selection in Vmax™ X2

| Antibiotic marker | Concentration | |
|---|---|---|
| | **Solid media** | **Liquid culture** |
| **Ampicillin** | 10–50 µg/mL | 50–100 µg/mL |
| **Carbenicillin** | 2–25 µg/mL | 5–100 µg/mL |
| **Kanamycin*** | 100 µg/mL | 400 µg/mL |
| **Tetracycline** | 10 µg/mL | |
| **Chloramphenicol** | 5–12.5 µg/mL | 12.5–25 µg/mL |

* Vmax™ X2 has a natural resistance to Kanamycin, resulting in a higher working concentration when using this antibiotic. Perform selection of kanamycin-resistant (KanR) colonies on agar plates with 100 µg/mL kanamycin and in liquid media with 400 µg/mL kanamycin for optimal results.

## Vmax™ X2 compatible plasmid origins of replication

A variety of commonly used plasmid origins of replication and antibiotic selection markers are known to be compatible with Vmax™ X2. The optimal recovery temperature can vary with different combinations of origins of replication and antibiotic selection markers used. As shown in table 4, most combinations of post-transformation growth on agar plates can be performed at either 30°C or 37°C.

*When using kanamycin selection, always grow Vmax™ X2 Cells on LB-Miller agar plates at 30°C.*
The following table shows different plasmid origins of replications tested. If you do not know the origin of replication for the plasmid you are using, we recommend performing duplicate reactions and plating cells for growth at both 30°C and 37°C or determining plasmid copy origin using an online resource such as wishart.biology.ualberta.ca/PlasMapper.

### Table 4. Transformation guidelines for Introducing plasmid into Vmax™ X2

| Plasmid origin of replication | Plasmid backbone | Compatible with Vmax™ X2 | Growth plate recommendation | |
|---|---|---|---|---|
| | | | Selection with kanamycin | Selection with all other antibiotics except kanamycin |
| **pMB1** | pBR322<br>pET vectors | Yes | 30°C only | 37°C recommended<br>(30°C acceptable) |
| **ColE1** | pCDNA3.0<br>pGEM5zf | | | |
| **pUC** | pUC19 | | | |
| **p15A** | pACYC184 | | | |
| **RK2** | N/A | Maybe | Unknown compatibility with chemical transformation. Can be introduced by conjugation. | Unknown compatibility with chemical transformation. Can be introduced by conjugation. |

# Protocols

## Before starting

If this is your first time using Vmax™ X2 Chemically Competent Cells, we recommend reading through the entire protocol before starting. While the Vmax™ X2 workflow is similar to that of E. coli, there are minor differences in the handling of Vmax™ cells that are important to understand prior to starting your work. Be sure to adhere to the guidance provided in the protocol for the best results.

## Materials

### Required materials provided

- Vmax™ X2 Chemically Competent Cells
- Vmax™ Chemicompetent Cell Recovery Medium
- Positive control pACYC/Chlor plasmid (optional)

### Materials and equipment not supplied

- Ice bucket
- Sterile culture tubes (e.g. Falcon® 14-mL round-bottom polypropylene tubes, Corning cat. no. 352006)
- Plasmid DNA for transformation (minimum concentration of 1 ng/µL in TE buffer or water)
- Water bath, pre-heated to 42°C
- LB-Miller Agar selection plates (e.g. Teknova cat. no. L1007)
- Antibiotic for plasmid selection (see table 3 on page 9 for recommended concentrations)
- Sterile spreader or sterile beads for plating cells on solid media
- Vmax™ Enriched Growth Medium (SGI-DNA cat. no. CL1500-1000) or other bacterial growth medium (see table 2 on page 8)
- Sterile glycerol and cryopreservation tubes (for creation of glycerol stocks for long-term storage)
- Incubators and orbital shakers set to appropriate temperature for recovery and growth
- Pipettors and plastics
- Baffled growth flask for large-scale protein expression
- IPTG
- Centrifuge and centrifuge tubes capable of > 4000 × g

## Heat shock transformation

1. For each transformation reaction, thaw one vial of Vmax™ X2 Chemically Competent Cells on ice for 5–10 minutes. If necessary, gently flick the tube to verify that the cells are thawed.
   Note: Use cells within 30 minutes of thawing.
2. Add 1–25 ng of plasmid DNA (in a volume of 1-5 µL, diluted in water or TE) directly into the competent cells. Gently flick the tube a few times to mix and place back on ice.
3. Incubate the DNA and cell mixture on ice for 30 minutes. For multiple transformations, place tubes into a water bath float while on ice for easy movement between temperatures.
4. During incubation, pipette 1 mL of Vmax™ Chemicompetent Cell Recovery Medium to a 14-mL round-bottom tube. Prepare a separate tube for each transformation reaction.
5. Place tubes in a rack and prewarm by using a 37°C water bath or incubator until needed in step 8.
6. After 30 minutes on ice, transfer the cells to a 42°C water bath for 45 seconds without shaking.
7. Transfer the cells back to ice for 1.5 minutes.
8. At the end of 1.5 minutes remove the cells from ice and place on benchtop. Pipette ~500 µL of prewarmed media from the 14 ml tubes into the transformation reaction, then transfer the mixture of media and cells back into the original 14 mL tube. Progress sequentially until all of the reactions have been recovered.
9. Place the 14 mL tubes in an orbital shaker (225 rpm) at 37°C for 2 hours. Shortening this step will decrease transformation efficiency. Proceed to *Plating* after recovery.
10. During the recovery step, prewarm selective LB agar plates at 37°C.

## Plating

### Important notes

- Vmax grows very well at a variety of temperatures, from room temperature up to 37°C (25°C<30°C<37°C). This allows for great flexibility in designing workflows and the ability to adapt to your lab's needs.
- For the highest transformation efficiencies and largest colony size, we recommend incubating plates at 37°C (except kanamycin plates must be incubated at 30°C).
- Colonies typically appear after 12–16 hours at 25°C to 30°C and after ~8 hours at 37°C.
- LB agar plates with established Vmax™ X2 colonies can be stored at room temperature for at least ten days. During this time, Vmax X2 colonies will remain well-spaced and can be used for inoculation or re-streaking onto fresh plates.
- LB agar plates containing Vmax™ cells should not be stored at 4°C, as this can adversely impact repropagation and growth.
- For long-term storage, we recommend preparing glycerol stocks with 25% glycerol and storing at −80°C.
- Plasmid DNA isolated from *E. coli* generally displays a tenfold decrease in transformation efficiency as compared to the same plasmid DNA isolated form Vmax cells.
- Standard *E. coli* miniprep protocols are compatible with Vmax™ cells.

### Plating protocol

1. We recommend trying (2) plating schemes when establishing a new vector with Vmax Rapid Express cells. For Vmax-derived DNA, plate 1 µL & 10 µL in a total volume of 50-100 µL. For DNA purified from *E. coli,* we suggest plating 5 µL & 50 µL in a total volume of 50-100 µL. Use either sterile beads or a spreader to evenly distribute cells across the surface of the plate.
2. *(Optional)* For the control plasmid, dilute the transformation reaction 1:250 with Vmax™ Chemicompetent Cell Recovery Medium. Plate 100 µL of the diluted reaction on an LB agar plate supplemented with 5–12.5 µg/mL chloramphenicol.
3. Incubate plates at 30°C or 37°C overnight, with visible colonies after 6–8 hours.
   Note: For kanamycin-based plasmids, only incubate at either 25°C to 30°C.

## Protein expression protocol

### Before starting

- The extremely fast growth rate and nutrient flexibility of Vmax™ X2 cells provides several advantages over *E coli*, including significantly faster iterations through more conditions and timepoints. We suggest that exploring these possibilities early in the evaluation phase on a smaller scale allows for ideal determination of conditions, including expression temperature and time (e.g. compare 4–6 hours and 18–24 hours).
- Vmax can be induced at <<0.5 OD. There's no need to closely monitor the culture prior to induction. This equates to shorter incubation times after inoculation allowing for same-day evaluation of expression.
- We recommend storing protein expression media at room temperature and adding antibiotics as the need arises.
- For optimal results, we recommend using Vmax™ Enriched Growth Medium (SGI-DNA cat. no. CL1500-1000) or enhanced 2xYT medium (see "Media recipes" on page 16). Refer to table 2 on page 8 for media options.
- Add appropriate antibiotics at required concentrations to the media for maintaining plasmid selection (refer to table 3 on page 9 for suggested concentrations).

### Rapid (small-scale) expression protocol

You may begin the Rapid Expression protocol the following morning after colonies appear from transformation the day before. Alternatively, we recommend morning inoculations from an established glycerol stock on the smaller scale as well.

1. Add 5–15 mL of Vmax Enriched Growth Medium to a disposable 50 mL Falcon tube. Add appropriate antibiotic and inoculate with a colony off an agar plate (or from glycerol stock).
2. Grow for 2–3 hours at 30°C while shaking at 225 rpm, then split culture in half. Maintain one culture as an uninduced control for analysis and glycerol stock. Induce the other culture with IPTG, then return both to 30°C incubator.
3. Grow throughout the day at 30°C and 225 rpm. We recommend taking samples at several timepoints to determine best conditions for expression if this is the first time expressing in Vmax.
4. For each sample, remove 500 µL from each culture and spin down for 5 minutes at 3000 x g.
5. Draw off supernatant and save pellet for analysis. Proceed directly to cell lysis or store at -80°C. If desired, save some of the uninduced culture for a glycerol stock. Remove 750 µL, mix 1:1 with 50% glycerol, and store at -80°C.
6. Continue growing culture overnight.
7. The next morning, process samples for an overnight timepoint and proceed to PAGE analysis.
   Note: Vmax X2 cells are compatible with standard *E. coli* lysis protocols (chemical and mechanical), allowing for simple examination of total and soluble protein expression. Once you have determined the ideal conditions for expression on a smaller scale, move on to large scale expression.

## Large-scale expression protocol

### Prepare small overnight culture

1. Add 2–5 mL of Vmax Enriched Growth Medium to a 14 mL culture tube.
2. Add appropriate volume and concentration of antibiotic (table 3).
3. Inoculate media with a colony from transformation or glycerol stock.
4. Incubate overnight at 30°C at 225 rpm.

### Large-scale growth

1. Remove inoculation culture from incubator.
2. Obtain a sterile, baffled flask (250 mL–2 L) or fermenter. Add Vmax Enriched Growth medium up to ¼ flask volume, then add appropriate antibiotic.
3. Using overnight culture, inoculate fresh media 1:1000 and grow for one hour at 30°C and 225 rpm.
   Note: Expression can be performed at 37°C, but for most proteins tested, expression is optimal when cells are grown at 30°C on a rotating shaker incubator at 225 rpm.
4. Following the inoculation approach in step 3, the $OD_{600}$ should be ≥ 0.1. If desired, measure the $OD_{600}$ for induction at 0.5. In the recommended Vmax Enriched Growth Medium, Vmax grows ~2.5-fold every 30 minutes between an $OD_{600}$ of 0.1 to 1.0.
   Note: Vmax™ X2 cells can be induced within a wide range of time points during growth phase. The optical density at induction can be between 0.1–1.0 OD600 with no apparent difference in expression. If auto-induction medium is used, expression will initiate naturally as the culture expands.
5. Induce 1:1000 with 1M IPTG and grow at 25°C or 30°C for 4–24 hours.
6. Harvest cells by transferring to a centrifugation tube and spinning at 4000–5000 × g for 10 minutes at room temperature. Carefully remove supernatant.
7. Store the cell pellet at −80°C or proceed to lysis and protein purification using preferred protocol.

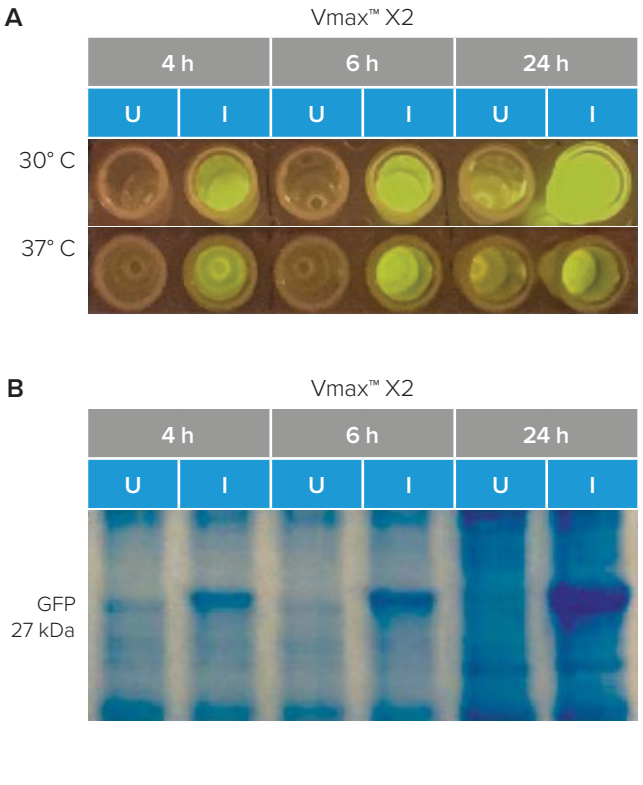
## Glycerol stocks

After growing an uninduced culture for a period of 6–8 hours, prepare a glycerol stock for long-term storage.

1. Add 750 µL of sterile 50% glycerol to a cryopreservation vial, then add 750 µL of Vmax culture from the uninduced culture. Invert tube several times to mix.
2. Place vials in –80°C freezer for storage.
3. To recover culture, use a sterile inoculation loop to scoop frozen culture and transfer to liquid or solid growth medium with appropriate antibiotics and incubate at room temperature, 30°C, or 37°C.

# Appendix

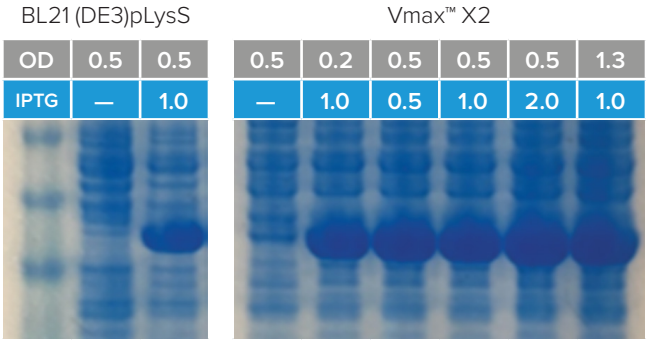
## Vmax™ X2 exhibits high protein expression




### Figure 2. High levels of protein expression in Vmax™ X2 over time in 4–24 hours post-induction.

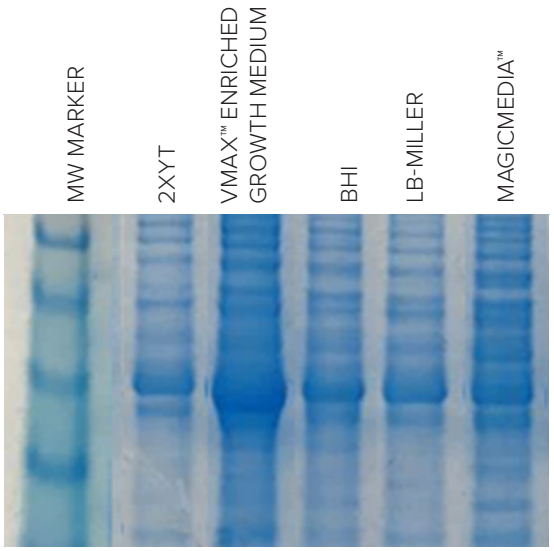
**(A)** To evaluate protein expression over time, a GFP expression plasmid was introduced into Vmax™ X2 cells. Expression was performed at 30°C and 37°C and analyzed at 4-, 6-, and 24-hour time points. Images of open culture tubes showing the luminescence of GFP in induced cells (I) and uninduced cells (U) under UV lamp are shown. **(B)** Gel analysis of proteins from uninduced and induced Vmax™ X2 cells grown at 30°C. **(C)** To evaluate protein expression in Vmax™ X2 and *E. coli*, uronate dehydrogenase (UDH) or aldose 1-epimerase expression vectors were introduced into *E. coli* BL21(DE3)pLysS and Vmax™ X2. The two metabolic proteins are more highly expressed in Vmax™ X2 and demonstrate significant stability over 24 hours of expression. In all panels shown, expression was analyzed by the amount of soluble protein in equal volumes of culture at the indicated time points.

## Vmax™ X2 supports induction over a wide range of optical densities and media




### Figure 3. Vmax™ X2 supports induction over a wide range of optical densities and can yield greater amounts of soluble protein.

Vmax™ X2 and *E. coli* BL21(DE3)pLysS cells containing an expression construct for uronate dehydrogenase were used for protein expression. BL21 cells were grown in LB medium at 37°C to an OD of 0.5 and induced with 1 mM IPTG and grown for an additional 4 hours. Vmax™ X2 cells were grown in Vmax Enriched Growth Medium at 30°C to variable ODs and induced with 0.5, 1, and 2 mM IPTG and grown for an additional 4 hours. Vmax™ X2 cells generate more soluble protein per cell and greater biomass.




### Figure 4. Vmax™ X2 exhibits high expression levels using different types of growth media.

Vmax™ X2 containing an expression construct for aldose 1-epimerase were cultured in 2xYT (lane 1), Vmax Enriched Growth Medium (lane 2), LB (lane 3), BHI (lane 4), and MagicMedia™ *E. coli* Expression Medium (lane 5). After two hours of growth, cells were induced with 1 mM IPTG.

## Media recipes

| Media | Contents |
|---|---|
| **LB-Miller** | 10.0 g/L Tryptone<br>5.0 g/L Yeast extract<br>10.0 g/L NaCl |
| **LB-Miller + v2 salts** | LB broth supplemented with additional salts:<br>204.0 mM NaCl<br>4.2 mM KCl<br>23.14 mM $MgCl_2$ |
| **LB-Miller agar** | LB-Miller broth + 1.5% agar |
| **Brain heart infusion broth + v2 salts** | 37 g/L Brain Heart Infusion Broth Dry Media (Teknova Cat. No. B9500) supplemented with additional salts:<br>204.0 mM NaCl<br>4.2 mM KCl<br>23.14 mM $MgCl_2$ |
| **Brain heart infusion + v2 salts agar** | Brain Heart Infusion Broth + v2 salts + 1.5% agar |
| **Terrific broth** | 12 g/L Tryptone<br>24 g/L Yeast extract<br>4 g/L Glycerol<br>1X Phosphate buffer (17 mM $KH_2PO_4$, 72 mM $K_2HPO_4$) |
| **2xYT media** | 16 g/L Tryptone<br>10 g/L Yeast extract<br>5 g/L NaCl |
| **Enhanced 2xYT medium** | 20 g/L Yeast extract<br>32 g/L Tryptone<br>17 g/L NaCl supplemented with 0.2% glucose and 17.6 mM $Na_2HPO_4$ adjusted to pH 7.4 |

## Troubleshooting

| Observation | Potential cause | Recommendation |
|---|---|---|
| **Few or no colonies** | Procedural issues | • Use provided control to verify transformation. Vmax™ X2 cells should exhibit transformation efficiencies of at least $1x10^7$ with the supplied control.<br>• After transformation, recover for two hours.<br>• Plate entire transformation reaction. |
| | Transformation efficiency may be dependent on the vector backbone or construct | • Ensure you are using a compatible plasmid origin of replication and antibiotic selection marker. Follow the guidelines in "Table 4. Transformation guidelines for introducing plasmid into Vmax™ Express" on page 10 for best results.<br>• Transformation efficiencies are typically tenfold higher with Vmax™-isolated vectors than with *E. coli*-derived vectors. For *E. coli*-derived vectors, plating a larger amount of transformation mix typically yields a sufficient number of colonies for expression. |
| | Use of inappropriate media or antibiotic | • Verify that you are using LB-Miller plates with the appropriate amount of salt. Do not use low salt LB-Lennox or LB-Luria plates.<br>• Ensure that you are using antibiotics at the recommended concentrations. Prepare fresh stocks of materials if needed. |
| | Improper handling or heat shock | • Prior to completion of the recovery step, handle cells gently. Avoid pipetting up-and-down to mix. Gently tap tube to mix contents.<br>• Verify that cells are incubated in 42°C water bath for 45 seconds, then immediately placed back on ice and allowed to sit undisturbed for 1.5 minutes before pre-warmed recovery media is added. |
| **Heterogeneous colony size** | Vmax™ cells have a natural resistance to kanamycin and are more sensitive to ampicillin/carbenicillin | • Use recommended antibiotic selection concentrations listed in "Table 3. Maintaining antibiotic selection in Vmax™ X2" on page 9. |
| **Lawn of colonies** | Issue with antibiotic selection | • Verify that you are using the correct antibiotic at the recommended concentration. See "Table 3. Maintaining antibiotic selection in Vmax™ Express" on page 9.<br>• Prepare fresh antibiotics and agar plates. |

| Observation | Potential cause | Recommendation |
|---|---|---|
| **No or poor growth in liquid culture** | No viable colonies on plate due to improper Vmax™ X2 storage | • Store Vmax™ X2 clones on solid media at room temperature for up to 10 days. Do not store Vmax™ X2 cells on solid media at 2°C to 8°C. For long-term storage, maintain clones in glycerol stocks. |
| | Antibiotic concentration is too high | • The minimal inhibitory concentrations of antibiotics for Vmax™ X2 are different from E. coli. Use recommended concentrations listed in "Table 3. Maintaining antibiotic selection in Vmax™ Express" on page 9. |
| | Expressed protein may be toxic to cells | • Codon optimization may be required.<br>• Contact SGI-DNA technical service for further recommendations on optimal construct design. |
| **No or low protein expression** | Expressed protein may be not be soluble | • Express protein at reduced temperature.<br>• Express protein for shorter duration.<br>• Induce expression at an earlier or later time point. |
| | Suboptimal culture condition | • Vmax™ X2 is compatible with many types of commercially available media. However, protein expression levels may vary between different formulations. Refer to "Table 2. Vmax™ X2 growth conditions in different media" on page 8. |
| | IPTG concentration too low or IPTG stock has reduced activity | • Use IPTG at a final concentration of 1 mM.<br>• Repeated freeze thawing results in reduced IPTG efficacy.<br>• Use a fresh aliquot or newly prepared IPTG. |

## References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