VMAX™ X2 CHEMICALLY COMPETENT CELLS

SGIDNA

Quick reference manual for experienced users | Catalog numbers CL1300-05, CL1300-10, CL1300-20

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

Vmax™ X2 handling practices

- Store cells immediately upon arrival at -80°C and use within six months of receipt. Protect cells from temperature fluctuations during storage.
- Thaw cells on ice just prior to use. Gently flick tubes to mix or resuspend. Avoid vortexing cells.
- Store Vmax™ Chemicompetent Cell Recovery medium at room temperature and use proper sterile techniques to avoid contamination.
- After transformation, recover cells for two hours only with Vmax™ Chemicompetent Cell Recovery medium. Do not use SOC or other recovery media.
- Vmax grows very well at 25°C<30°C<37°C, allowing for flexibility in designing workflows. However, transformation reactions on LB agar plates with kanamycin must be incubated O/N at 30°C or 25°C.
- Vmax LB agar plates can be kept at room temperature for at least ten days, during which time colonies may be used for inoculation and/or re-streaking onto fresh plates. Do not store at 4°C.
- For long-term storage, prepare glycerol stocks and store at -80°C.

Notes:

- Vmax can be induced early in growth phase and does not require growth to OD_{600}= 0.5 for induction with IPTG.
- Plasmid DNA isolated from Vmax cells displays a tenfold higher TE than the same vector isolated from *E. coli*.

Antibiotic marker	Solid media	Liquid culture
	Concentration	
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	10 µg/mL
Chloramphenicol	5–12.5 µg/mL	12.5–25 µg/mL

Heat shock transformation protocol

1. Thaw Vmax™ X2 Chemically Competent Cells on ice for 5–10 minutes.
2. Add 1–25 ng of 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 cells on ice for 30 minutes.
4. During the 30-minute incubation, pipette 1 mL of Vmax Chemicompetent Cell Recovery medium into a Falcon® 14-mL round-bottom tube. Prepare a separate tube for each transformation reaction.
5. Place tubes into a rack and pre-warm 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 Falcon tubes into the transformation reaction, then transfer the mixture of media and cells back into the original 14 mL Falcon 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 two hours. Shortening this step will decrease transformation efficiency.
10. During the recovery step, pre-warm selective LB agar plates at 37°C.

Glycerol stocks

1. After growing an uninduced culture for a period of 6 to 8 hours, prepare a glycerol stock for long-term storage.
2. Add 500 µL of sterile 50% glycerol to a cryopreservation vial, then add 500 µL of Vmax culture from the uninduced culture. Invert tube several times to mix.
3. Place vial(s) in -80°C freezer for storage.

Plating instructions

1. We recommend trying (2) plating schemes when establishing a new vector with Vmax X2 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 on 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. Colonies can be visualized after 6–8 hours of incubation.

Note: For kanamycin-based plasmids, only incubate plates at either 25°C or 30°C.

Rapid protein expression protocol

Guidelines for smaller scale protein expression and initial screening

1. Add 3 mL of Vmax Enriched Growth Media to a a disposable 50 ml Falcon tube. Add appropriate antibiotic for protein expression vector (see antibiotic chart for guidelines).
2. Inoculate media with a colony from plate. Grow culture at 37°C for ~2 hours (visible turbidity) on a rotating shaker incubator at 225 RPM.
3. Transfer half the culture to a new tube for an uninduced control for gel analysis and glycerol stock (see “Glycerol stocks”), once this culture has grown for 6–8 hours.
4. Add 5 µL of 1M IPTG to the test culture for induction. Return cultures to either 25°C or 30°C shaking incubator.
5. Incubate induced cells for 4–24 hours and harvest using preferred method.

Larger-scale protein expression protocol

Prepare small overnight culture

1. Add 3–5 mL of Vmax Enriched Growth Media to a 14 mL Falcon tube.
2. Add appropriate volume & concentration of antibiotic (see chart).
3. Inoculate media with a colony from transformation.
4. Incubate overnight at 30°C at 225 RPM.

Large-scale growth

1. Remove inoculation culture from incubator.
2. Obtain sterile, baffled flask (250 mL–2 L) or fermenter. Add Vmax Enriched Growth media 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 while shaking at 225 rpm.
4. Vmax X2 can be induced at OD_{600} 01. to 1.0.
5. Induce 1:1000 with 1M IPTG and grow at 25°C or 30°C for 4 to 24 hours.
6. Process the induced culture for overexpressed protein of interest using preferred protocol.

Recipes

For optimal cell growth in liquid cultures, Vmax™ Enriched Growth Medium (SGI-DNA cat. no. CL1500-1000) is recommended for best results. Alternatively, the following liquid media can be used. Using any other liquid media can result in poor growth and/or expression.

For solid media, we recommend LB agar plates (Miller formulation), made using the below recipe or purchased with the above antibiotic concentrations.

Liquid media

LB + V2 salts
LB Miller media, supplemented with additional salts:
204.0 mM NaCl
4.2 mM KCl
23.14 mM MgCl2

Enhanced 2xYT
20 g/L yeast extract
32 g/L tryptone
17 g/L NaCl supplemented with 0.2% glucose and 17.6 mM Na2HPO4 adjusted to pH 7.4

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