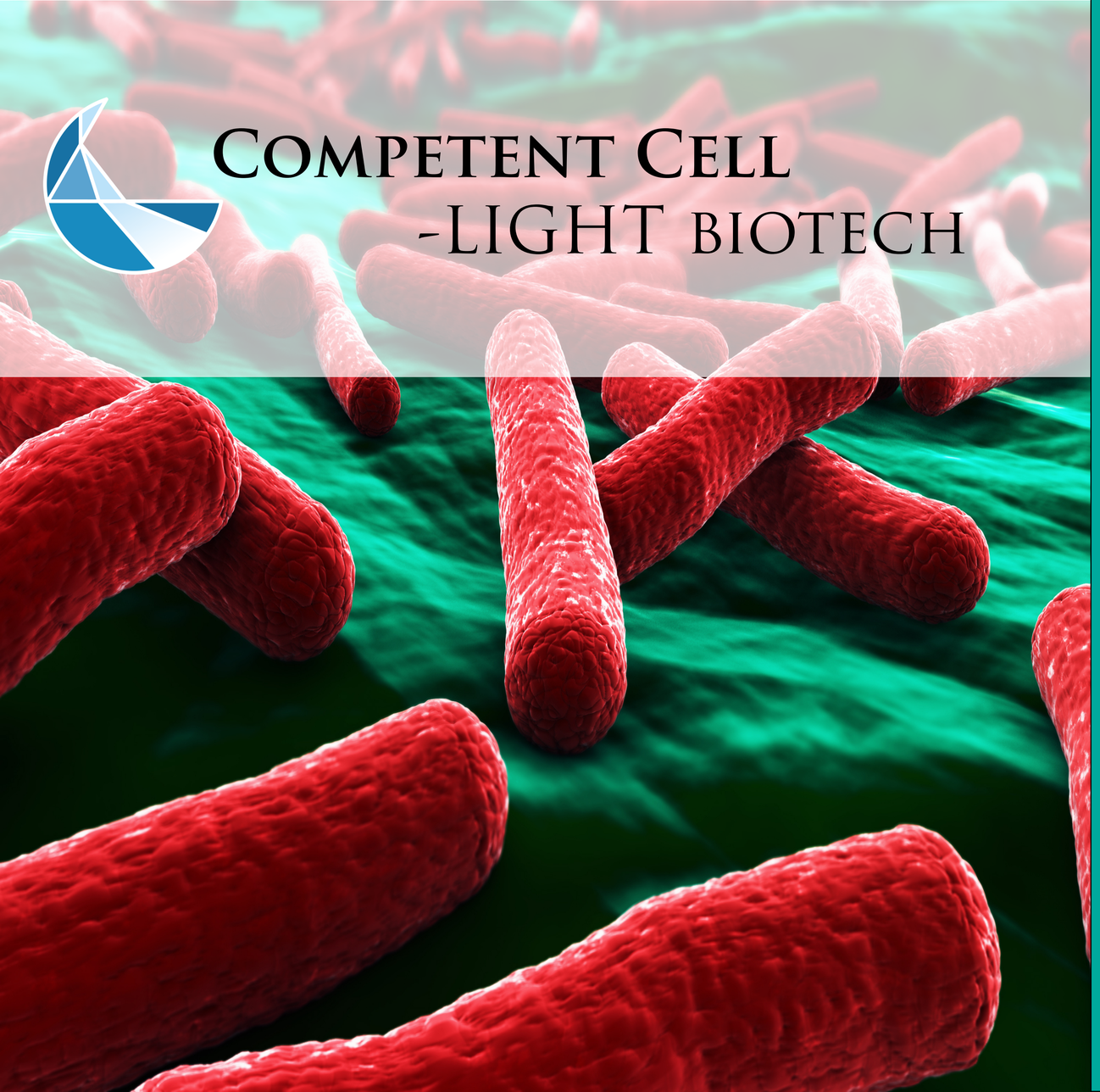


Competent Cell -LIGHT biotech

Catalogue

General Information

Competent Cell -LIGHT biotech are chemically competent cells, which were prepared using our proprietary process to make the cells highly efficient for immediate DNA uptake. The transformation process only takes 1 minute to finish and the steps are simplified.

Shipping Conditions

Throughout the shipping process, two electronic temperature recorders are included in the package to monitor the shipping temperature at all times.
The shipping temperature is maintained within at -65 ± 5℃.

Storage and Expiration

Competent Cell -LIGHT biotech must be stored at -70℃ to -80℃. Subsequent freeze-thaw cycles will reduce transformation efficiency. If high efficiency is essential for the experiment, do not use aliquots that have gone through several freeze-thaw cycles.
The efficiency of Competent Cell -LIGHT biotech is good for 1 year with proper storage.

Items and Ordering Information

Cat. No.	Product Name	Compatible to (strain)	Efficiency (cfu/ μg)	Quantity
D108-100	DH5α Competent Cell -LIGHT biotech	E. coli DH5α	> 3×10^8	100 μl x 100 vials
J108-100	JM109 Competent Cell -LIGHT biotech	E. *coli* JM109	> 1×10^8	100 μl x 100 vials
B107-80	BL21 Competent Cell -LIGHT biotech	E. *coli* BL21	> 5×10^7	100 μl x 80 vials

Genotypes and Applications

Product	Genotype	Application
DH5α Competent Cell -LIGHT biotech	*end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 Δ(*lac*ZYA-*arg*F)U169 Φ80Δ(*lac*Z)M15 F-	Suitable for cloning with large plasmids and cDNA library construction, and also allow blue-white colony selection.
JM109 Competent Cell -LIGHT biotech	*e*14^-(*Mcr*A^-) *rec*A1 *end*A1 *gyr*A96 *hsd*R17(r_k^-, m_k^+) *sup*E44 *rel*A1 Δ(*lac-Pro*AB) [F' *tra*D36 *pro*AB *lac*I^qZΔM15]	Appropriate for blue-white color and robotic screening. It is a fast growing strain, forming visible colonies within 8~10 hours.
BL21 Competent Cell -LIGHT biotech	F' *omp*T *hsd*S_β(r_β^- m_β^-) dcm gal λ(DE3)	Appropriate host for recombinant protein expression using T7-based expression vectors.

1 Minute Transformation Protocol
Heat shock / cold plating

Thaw competent cells (typically, 100ul) at room temperature in water bath with circulating water or holding the tube under the running tap water for ~20 seconds until 1/3~1/2 volume is thawed. (If many transformations are carried out at once and step 2 cannot be done immediately, keeping the competent cells on ice will generate better efficiency.)

Add DNA (pre-chilled on ice, volume should be ≦ 5% of competent cells) immediately. Vortex for 1 second or tap the tube with finger to mix well. (Optional step (3): 2~6 minute protocol): Keep the tube on ice for 1~5 min will increase the efficiency a little bit. If many transformations are carried out at one time, this optional step will facilitate the process and make the overall efficiency more uniform.)

Heat shock the cells in water bath at 42°C for 15~45 seconds. (For optional heat shock durations, please refer to Q8.)

Plate the cells onto a pre-chilled (4°C) and dried selective LB agar plate (LB + antibiotics).

Incubate the plates at 37°C (8~16 hours for JM109 Competent Cell -LIGHT biotech, 12~16 hours for others).

Non-heat shock/ warm plating

1. Prewarm a selective LB agar plate at room temperature up to 37°C incubator.

2. Thaw competent cells (typically, 100ul) at room temperature in a water bath with circulating water or holding the tube under the running tap water for ~20 seconds until 1/3~1/2 volume is thawed. (If many transformations are carried out at once and step2 cannot be done immediately, keeping the competent cells on ice will generate better efficiency.)

3. Add DNA (pre-chilled on ice, volume should be ≦ 5% of competent cells) immediately Vortex for 1 second or tap the tube with finger to mix well. (Optional step (4): 2~6 minute protocol); Keeping the tubes on ice 1~5 min will slightly increase the efficiency. If many transformations are carried out at a time, this optional step will facilitate the process and make the overall efficiency more uniform.

4. Plate the cells onto the pre-warmed and dried selective LB agar plate (LB + antibiotics).

5. Incubate the plates at 37°C (8~16 hours for JM109 Competent Cell -LIGHT biotech, 12~16 hours for others).

Procedure Graph of 1 Minute Protocol

Notices for using Competent Cell -LIGHT biotech :

Ampicillin (Ap)	**20 µg/ml**
Kanamycin (Km)	**25 µg/ml**
Tetracycline (Tc)	**7.5 µg/ml**
Chloramphenicol (Cm)	**20 µg/ml**

If the antibiotic concentration is out of the range or kinds of antibiotics are used, It will reduce the transformation efficiency distinctly.

Circulating water bath or running tap water until ≅ ½ competent cell thawing.

Incubate on ice, add pre-chilled plasmids.

Vortex 1 sec

Next page

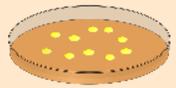
Total Time : 1 minute

Protocol 1

For plasmids < 6 kb

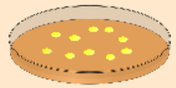
42˚C

Heat shock 45 sec.



Plating on 4˚C cool or RT, dry plate. Incubation at 37˚C.

Protocol 2



Plating on 37˚C dry plate. Incubation at 37˚C.

* The efficiency level of these four protocol ranks as 3>1>4>2.

Total Time : 6 minute

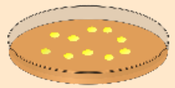
Protocol 3

For low copy, large plasmids or BACs. For kanamycin selection.

Incubate on ice 5 min.

42˚C

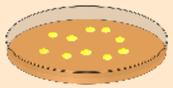
Heat shock 45 sec.



Plating on 4˚C cool or RT, dry plate. Incubation at 37˚C.

Protocol 4

Incubate on ice 5 min.



Plating on 4˚C cool or RT, dry plate. Incubation at 37˚C.

Quality Control

1. Efficiency test: Each batch of Competent Cell -LIGHT biotech is checked rigorously to meet the product specifications as described in "Item and Ordering Information" at the time of production.
   Calculation of transformation efficiency is as follows:

   a. Equation for transformation efficiency = transformed colonies (transformants) / µg of plasmid.

   b. Example:
   100µl of competent cells have been transformed with 10-6 µg of pUC19 plasmid. If 550 colonies are observed on the selective plate. The transformation efficiency is:
   550/10-6 = 5.5 x 10^8 transformants /µg of pUC19 plasmid.

2. Contamination test: Transformation is performed without plasmid. There should be no colony on the LB agar plate with 20~50 µg/ml of ampicillin.

3. α-complementation test for DH5α & JM109 Competent Cell -LIGHT biotech: To pass the test, the ratio of white colonies/ white & blue colonies of the tested plate should be less than 3%.

Q & A

Q1: How much will the efficiency be reduced if Competent Cell -LIGHT biotech are thawed, dispensed and refrozen repeatedly?

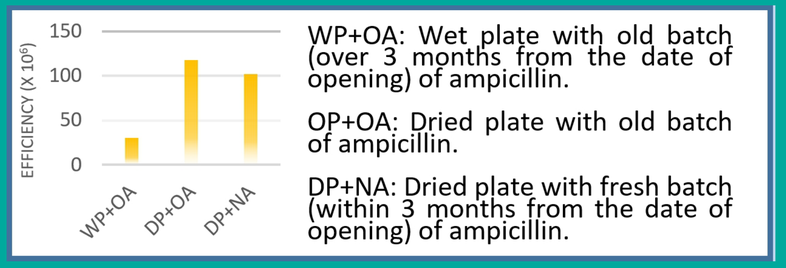
A1: If Competent Cell -LIGHT biotech are thawed, dispensed in aliquots and refrozen within 3 min, the transformation efficiency will be 20~50% less than first time use.

Q2: What's the advantage of thawing the cells with circulating water instead of still water?

A2: To thaw the cells with circulating water can increase the transformation efficiency by 1.5~3 times.

Q3: Do temperature, wetness of plating beads and plates affect transformation efficiency?

A3: The transformation efficiency is increased significantly when dried plating beads and plates are used.



Q4: Do the storage temperature and thawing method affect the transformation efficiency?

A4: Slow thawing caused by power shortage or unstable freezer will make the efficiency decreased. Therefore, it is very important that the competent cells are stored at -70°C at all time. Thawing the competent cells in water at room temperature yields better efficiency than thawing the cells on ice.

Q5: Is there a difference in transformation efficiency between using plating beads and streaking loop?

A5: Using plating beads gives higher transformation efficiency than using streaking loop.

Q6: How to prepare dried selection plates?

A6: During the process of making the agar plates, leave the freshly made plates in a laminar flow for 30~60 min to allow the moisture to be fully evaporated. Then store the plates at 4°C (chilling & dry) for more than 1 hour.

Q7: Is one second vortex before 42°C heat shock necessary for transformation?

A7: One second vortex leads to more reliable transformation efficiency (1.2 times higher than mixed by finger tapping).

Q8: Does the duration of heat shock affect transformation efficiency?

A8: There is little difference in transformation efficiency within 15~45 seconds of heat shock. For DH5α Competent Cell -LIGHT biotech 15~35 seconds of heat shock is optimal for transformation of plasmid < 6 Kb, 45 seconds heat shock is not optimal for plasmid < 6 Kb (may cause 1.2~2.5 times decrease) but is optimal for plasmid > 6 Kb.

Q9: Is it necessary to change the transformation procedure for transforming E. coli with a large plasmid?

A9: For large plasmid (>6 Kb), 6 min protocol should be used to significantly improves the efficiency (Table 2).

Q10: How to reduce the interference of the satellite colonies?

A10: Using dried plating beads and plates with proper antibiotics at suitable concentrations. Fresh antibiotics are recommended.

Q11: Does the concentration of antibiotic in LB medium affect transformation efficiency?

A11:
For ampicillin :

DH5α Competent Cell -LIGHT biotech
LB + 20μg/ml fresh Amp. or 50μg/ml old Amp. that has been stored for more than 3 months. If higher concentration is used, e.g. 50~100 μg/ml fresh Amp., the efficiency will be 3~10 times lower. Transformed colonies can be observed after 11~16 hours of incubation and the satellite colonies will start to appear after 18 hours.

JM109 Competent Cell -LIGHT biotech
LB + 20μg/ml fresh Amp. or 50μg/ml old Amp. that has been stored for more than 3 months. If higher concentration is used, e.g. 50~100 μg/ml fresh Amp., the efficiency will be 3~50 times lower. Transformed colonies can be observed after 8~10 hours of incubation and the satellite colonies will start to appear after 24 hours.

(2) For other antibiotics:

LB + 20μg/ml Kanamycin, 7.5μg/ml Tetracycline, 20μg/ml Chloramphenicol. For plasmid size < 6 Kb, the efficiency of kanamycin selection is usually 3~10 times less than that of ampicillin selection.

For plasmid size > 6 Kb, the efficiency of kanamycin selections is much lower than that of ampicillin, We recommend using the traditional protocol (with the recovery step) to enhance the efficiency. For more information about the relationship of antibiotic concentrations, protocol used, and efficiency levels, check the experimental data in table 1 and 2.

Q12: Does the size of plasmid affect transformation efficiency?

A12: The size of plasmid affects the efficiency greatly. For instance, the efficiency for a supercoiled 2.7 Kb and a 10 Kb plasmid (using 1 minute protocol) is 1.6~5.5 x 109 and 4.0~9.0 x 10^6, respectively. The difference is approximately 100 times. Transformation efficiency for large plasmids (especially for > 6 Kb) can be increased by using 6 min protocol as described in Q9.

Q13: How to perform blue/ white screening?

A13: Please make sure the plasmid, which have been transformed to DH5α Competent Cell -LIGHT biotech, contains the LacZ operon. After transformation, please spread DH5α Competent Cell -LIGHT biotech onto LB Plates (containing 0.5 mM IPTG and 40~60 μg/ml X-gal). After incubation at 37°C, white colonies indicate insertion of foreign DNA on LacZ operon, and blue colonies indicate no insertion leading to functional β-galactosidase activity to hydrolyze the X-gal.

Table 1. The efficiencies under various conditions including plasmid size, antibiotics concentrations by 6 min protocol.

- If kanamycin is used for selection and the plasmid size > 6 Kb, we suggest using an improved recovery protocol, in which 0.4~0.9 ml LB broth is added to the cells followed by shaking at 37°C for 20~60 min before plating.

BG: Background (pseudo antibiotics resistants)

		Ampicillin (μg/ml)		Chloramphenicol (μg/ml)		Kanamycin (μg/ml)				Tetracycline (μg/ml)		
Product	Plasmid (size)	20	50	20	30	10	15	20	25	7.5	15	25
DH5α Competent Cell -LIGHT biotech	pUC19 (2.7 Kb)	1.4×10^9	9.2×10^8									
	pUC4k (4.0 Kb)	9.6×10^8	8.6×10^8			BG	9.2×10^7	3.5×10^7	1.6×10^7			
	pBR325-KR (7.4 Kb)	3.0×10^8	2.8×10^8	4.0×10^8	2.5×10^8	BG	*	*	*	3.0×10^8	1.0×10^8	1.3×10^7
JM109 Competent Cell -LIGHT biotech	pUC19 (2.7 Kb)	4.6×10^8	3.5×10^8									
	pUC4k (4.0 Kb)	3.3×10^8	1.6×10^8			5.4×10^7	1.6×10^7	1.1×10^7	2.7×10^7			
	pBR325-KR (7.4 Kb)	7.8×10^7	5.6×10^6	1.3×10^8	1.2×10^8	BG	*	*	*	BG	1.1×10^8	3.0×10^7
BL21 Competent Cell -LIGHT biotech	pUC19 (2.7 Kb)	1.3×10^8	4.7×10^7									
	pUC4k (4.0 Kb)	1.2×10^8	3.2×10^7			BG	BG	1.6×10^7	6.0×10^6			
	pBR325-KR (7.4 Kb)	8.0×10^6	3.0×10^5	2.0×10^7	1.6×10^7	BG	*	*	*	2.0×10^7	1.8×10^7	6.0×10^5

Table 2. The test of DH5α Competent Cell -LIGHT biotech transformation efficiencies under various conditions including, transformation method, plasmid size, and antibiotics concentration.

- If kanamycin is used for selection and the plasmid size > 6 Kb, we suggest using an improved recovery protocol, in which 0.4~0.9 ml LB broth is added to the cells followed by shaking at 37°C for 20~60 min before plating.

Note: Please refer to "Procedure Graph of 1 Minute Protocol",
Cold, 1 min = method(2) ; Cold, 6 min = method (4) ;
Warm, 1 min = method(1) ; Warm, 6 min = method (3).

		pUC19 (2.7 Kb)				pUC4k (4.0 Kb)				pBR325-KR (7.4 Kb)			
		Warm		Cold		Warm		Cold		Warm		Cold	
Antibiotic	protocol	1min	6min	1min	6min	1min	6min	1min	6min	1min	6min	1min	6min
(concentration)		-1	-3	-2	-4	-1	-3	-2	-4	-1	-3	-2	-4
Ampicillin	(20 μg/ml)	5.5×10^8	5.6×10^8	9.2×10^8	1.4×10^9	2.9×10^8	4.2×10^8	7.8×10^8	9.6×10^8	1.3×10^8	1.2×10^8	1.8×10^8	3.0×10^8
	(50 μg/ml)	3.5×10^8	3.9×10^8	1.0×10^9	1.1×10^9	1.9×10^8	4.6×10^8	9.5×10^8	9.0×10^8	1.0×10^8	7.0×10^7	2.7×10^8	3.1×10^8
Kanamycin	(15 μg/ml)					1.3×10^8	1.4×10^8	7.3×10^7	1.0×10^8	*	*	*	*
	(25 μg/ml)					2.1×10^7	2.1×10^7	1.0×10^7	1.6×10^7	*	*	*	*
Tetracycline	(7.5 μg/ml)									8.0×10^7	8.5×10^7	1.1×10^8	1.1×10^8
	(15 μg/ml)									3.0×10^6	4.4×10^7	5.0×10^6	1.0×10^8
Chloramphenicol	(20 μg/ml)									7.5×10^7	9.7×10^7	1.8×10^8	2.4×10^8
	(30 μg/ml)									3.0×10^6	7.6×10^7	1.1×10^7	1.3×10^8

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