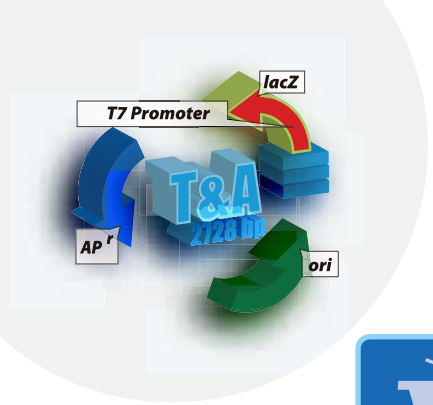
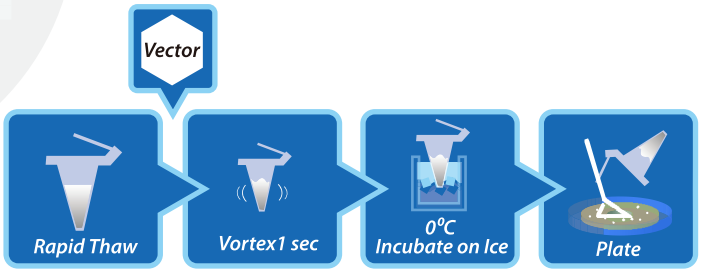


For reliable rapid cloning everytime , use RBC T&A Cloning Kit (Cat. No. RC001) !



10^7 to 10^9 Efficiency: High Ice Transformation

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HIT™ Competent Cells
Real Biotech Corporation

-70°C Electronic Monitoring

Non-Heat Shock Transformation: Protocol Book

HIT Competent Cells™

Contents

Product Description

Introduction

HIT Competent Cells™ (1 minute competent cells) are the fastest transforming cells worldwide. Due to our unique production process, cells can be transformed to high efficiency in 1-10 minutes using a simple 1 step patented protocol.

Contents

- ***HIT Competent Cells™***
- ***pUC19 Control Plasmid (10^{-4} µg/µl): 5 µl***
- ***Protocol Book.***

Shipping Conditions

HIT Competent Cells™ have been electronically temperature monitored to ensure -70°C AT ALL TIMES during batch processing, shipping and storage.

Storage Conditions

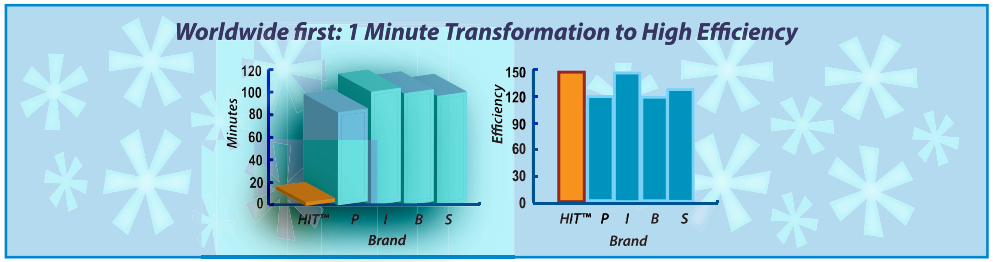
HIT Competent Cells™ should be stored immediately upon receipt at -70°C in a constant temperature freezer. HIT Competent Cells™ can be stored for up to 12 months without showing any deduction in performance and quality with proper storage.

Note: Please do not store HIT Competent Cells™ in liquid nitrogen.

Calculation of Transformation Efficiency

HIT Competent Cells™ transformation efficiency reaches 10^7~10^9 transformation / µg pUC19 plasmid DNA(varies according to strains and plasmid size).

Formula	transformation efficiency = (transformed colonies) / (µg of plasmid)
Example	8.2 x 10^8/ µg (efficiency) = 823 (transformed colonies) / 10^{-6} µg
Test for	RH619: HIT Competent Cells™-DH5a Super 10^9
Selection	LB agar (Ap 50 µg/ml)
Results	test with 10^{-6} µg pUC19 plasmid, resulted in efficiency of 8.2 x 10^8/µg



Application Table

Cloning Applications	HIT™-DH5a	HIT™-JM109	HIT™-Blue	HIT™-21	HIT™-DH10B	HIT™-GM2163
Large Plasmids > 6 kb*	Ideal	+	+	+	Yes	Yes
Subcloning	Ideal	Yes	Yes	+	Ideal	No
cDNA Library	Yes	Yes	Yes	+	Yes	+
Fast Growth	+	Ideal	+	Yes	+	+
Single Stranded DNA	+	Ideal	+	+	+	+
Toxic Protein Expression	No	No	No	No	No	No
Mutagenesis	Yes	+	+	No	Yes	+
Protein Expression	No	No	No	Ideal	+	No
Blue/White Screen	Yes	Yes	Ideal	No	Yes	No
DNA Unmethylation	No	No	No	No	No	Yes
Genomic DNA Cloning	No	No	No	No	Yes	No

* For high efficiency transformation of large plasmids (> subcloning efficiency), an alternative extended protocol is recommended. Please refer to FAQ.

\+ Indicates that the strain can be used for the purpose, but may not yield the best result.

HIT Competent Cells™

Genotypes Table

Genotypes	Applications	HIT™-DH5a	HIT™-JM109	HIT™-Blue	HIT™-21	HIT™-DH10B	HIT™-GM2163
		F-(80d lacZ M15) (lacZYA-argF)U169 hsdR17(r- m+) recA1 endA1 relA1 deoR	F' traD36 proA+ proB+ laclq (lacZ)M15 I (lac-proAB) hsdR17 recA1 endA1 relA1	hsdR17(rk- mk+), recA1, endA1, gyrA96, thi-1, supE44, relA1, lac[F' proAB laclqZDM15Tn10(Tet^r)]	E.coli B, F-, dcm, ompT, hsdS(rB-mB-), gal (DE3)	F- endA1 recA1 galE15 galK16 nupG rpsL ΔlacX74 Φ80lacZΔM15 araD139 Δ(ara,leu)7697 mcrA Δ(mrr-hsdRMS-mcrBC) λ-	F- ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrÅ dcm-6 hisG4 rfbD1 psL136 dam13::Tn9 xylA5 mtl-1 thi-1 mcrB1 hsdR2
endA1	Prevents plasmid degradation during extraction	Yes	Yes	Yes	No	Yes	No
recA1	Prevents DNA recombination	Yes	Yes	Yes	No	Yes	No
hsdR	Enhances transformation efficiency of selected PCR DNA strands and cDNA libraries	Yes	Yes	Yes	Yes	Yes	Yes
deoR	Enhances transformation efficiency of high MW plasmids and cosmids	Yes	No	No	No	Yes	No
LacZ M15	Inhibits LacZ gene expression for blue-white screen	Yes	Yes	Yes	No	Yes	No
Lon & ompT	Lon & ompT protease deficient and improves protein yield	No	No	No	Yes	No	No
rne131	Inhibits RNase E and improves mRNA stability	No	No	No	Yes	No	No
dam/dcm	Prevents DNA methylation	No	No	No	Yes / No	No	Yes
mcrA/mcrB	Prevents DNA methylated DNA from degradation	No	No	No	No	Yes	Yes

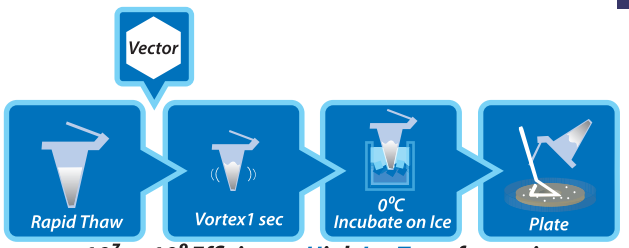
Non-Heat Shock Transformation Protocol (1-10 minutes, efficiency = 10^7~10^9/μg)

Please read the entire important notes listed in page 12 prior to starting any of the protocol procedures.

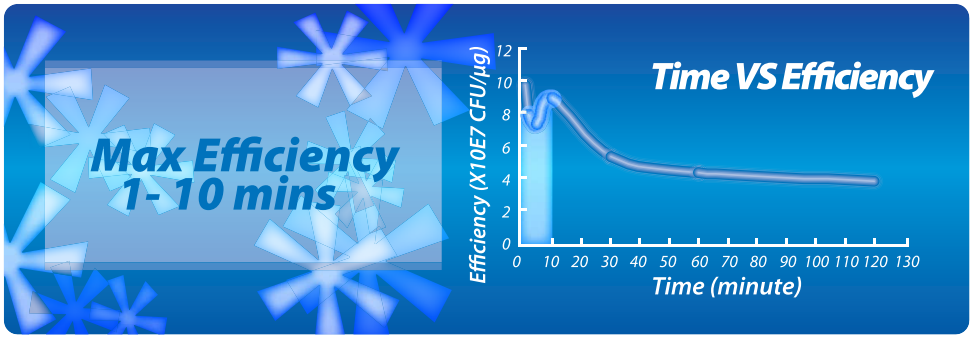
Attention: Prior to transformation, dry the plating beads. Agar plates shall be warmed to 37°C (A MUST).

Important: Complete the vortex step before there is still ice crystal left in the tube.

- Prepare ice bucket, 37°C plating beads and selective plates. Thaw entire competent cell vial with room-temp. tap water or water bath for 10~20 seconds until 1/3 thawed.
- Add DNA whose volume is less than 10% of volume of cells . Vortex 1 second.
- Place on ice for 1-10 minutes
- Transfer onto 37°C dry selection plate media, spread using RBC plating beads.
- Immediately incubate plate at 37°C (8-16 hours for JM109 strain, 16-18 hours for other strains: DH5a, DH10B, XL1-Blue, BL21 and GM2163). Observe growth of transformed colonies .



10^7 to 10^9 Efficiency: High Ice Transformation



Quality Control

Efficiency test and α-complementation test

- Thawing HIT Competent Cells™ with RT water for 20 seconds until 1/3 thawed.
- Add 1μl (10^{-6} μg/μl) pUC19 (ice-bathed plasmid).
- Vortex 1 second.
- Place on ice for 5 minutes.
- Using RBC plating beads for plating on LB plate which is with 50 μg/ml Amp.
- Immediately incubate the plate at 37°C for 16 hours.

Ampicillin-resistance test

- Thawing HIT Competent Cells™ with RT water for 20 seconds until 1/3 thawed.
- Vortex 1 second.
- Plating 50 μl cell on LB plate which is with 50 μg/ml Amp.
- Immediately incubate the plate at 37°C for 16 hours.
- Place the plate at RT for 3 days (Contamination test).

QC Reports : Now Online!

Each batch of HIT Competent Cells™ is rigourously checked for efficiency and other parameters at time of production. Go online to **WWW.REAL-BIOTECH.COM** and enter the LOT NO. of your HIT Competent Cells™ for a complete report. Each HIT Competent Cells™ shipment is -70°C electronically monitored and recorded for **best quality guarantee.**

Antibiotics Analysis Test

Strain	Plasmid	Ampicillin (μg/ml)		Chloramphenicol (μg/ml)		Kanamycin (μg/ml)				Tetracycline (μg/ml)		
		20	50	20	30	10	15	20	25	7.5	15	25
DH5a (Cat. No. RH619)	pUC19(size:2.7 Kb)	$1.7x10^9$	$9.1x10^8$									
	pUC4k (size: 4.0 Kb)	$9.8x10^8$	$8.5x10^8$			*	$9.5x10^7$	$3.7x10^7$	$1.5x10^7$			
	pBR325-KR(size: 7.4Kb)	$3.2x10^8$	$2.9x10^8$	$5.5x10^8$	$3.0x10^8$	*	*	*	*	$3.5x10^8$	$1.2x10^8$	$1.5x10^7$
JM109 (Cat. No. RH718)	pUC19(size:2.7 Kb)	$4.9x10^8$	$3.6x10^8$									
	pUC4k (size: 4.0 Kb)	$3.6x10^8$	$1.9x10^8$			$5.5x10^7$	$1.7x10^7$	$1.3x10^7$	$2.9x10^6$			
	pBR325-KR(size: 7.4Kb)	$7.9x10^7$	$5.8x10^6$	$1.5x10^8$	$1.4x10^8$	*	*	*	*	*	$1.4x10^8$	$3.2x10^7$
XL1-Blue (Cat. No. RH119)	pUC19(size:2.7 Kb)	$8.5x10^8$	$1.3x10^9$									
	pUC4k (size: 4.0 Kb)	$1.2x10^9$	$5.5x10^8$			$2.2x10^8$	$1.3x10^8$	$7.4x10^7$	$3.6x10^7$			
	pBR325-KR(size: 7.4Kb)	$2.1x10^8$	$2.3x10^8$	$2.0x10^8$	$1.6x10^8$	*	*	*	*			
BL21 (DE3) (Cat. No. RH217)	pUC19(size:2.7 Kb)	$1.5x10^8$	$4.8x10^7$									
	pUC4k (size: 4.0 Kb)	$1.4x10^8$	$3.3x10^7$			*	*	$1.5x10^7$	$6.2x10^6$			
	pBR325-KR(size: 7.4Kb)	$8.2x10^6$	$3.6x10^5$	$2.3x10^7$	$1.8x10^7$	*	*	*	*	$2.2x10^7$	$1.9x10^7$	$6.2x10^5$

* Not recommended.

HIT Competent Cells™

Important Notes

1. HIT Competent Cells™ provide best efficiency when cells are in about 1/3-volume thawed state (cells in completely thawed state will cause a 3-fold decrease in transformation efficiency).
2. Vortexing for 1 second will not affect the efficiency (HIT Competent Cells™ can stand high speed vortex).
3. Modified protocol for large plasmids (>6 kb) and cDNA libraries: 20 minutes ice bath followed by 1 minute 42°C water bath and another 20 minutes ice bath. Efficiency will increase 2 to 5-fold.
4. Further incubation with either SOC or LB medium is not required.
5. Plating using plating beads at 37°C and selective plates improves the transformation efficiency up to 3-fold when compared with room temperature plating beads.
6. The antibiotic concentrations are recommended as: Ap: 20~50 μg/ml; Km: 25 μg/ml, Tc: 7.5 μg/ml for HIT Competent Cells™-DH5a, DH10B, GM2163, JM109 and XL1-Blue strains. Higher antibiotic concentrations will decrease the efficiency. Lower concentrations will increase the number of satellite colonies.
7. Warning for HIT Competent Cells™-DH5a and HIT Competent Cells™-JM109 : Over incubation at 37°C for18-24 hours will result in satellite (pseudo-positive) colonies appearing.

FAQ

Q: For the transformation of larger plasmids, is it necessary to change the standard transformation procedure?

A: For the transformation of plasmids with higher molecular weight or cDNA libraries (vector + insert >6 kb), the standard procedure may be modified to a 20 minutes ice bath - 1 minute 42°C Heat Shock - 20 minutes ice bath protocol to increase transformation efficiency.

Q: Does the storage temperature and thawing method affect transformation efficiency?

A: HIT Competent Cells™ should be stored at -70°C~ -80°C condition at all time. Slow thawing caused by power cuts and unstable freezers will result in decreased efficiency. Thawing in room temperature water yields better efficiency than thawing on ice.

Q: Is there a difference between using plating beads and plating loop in terms of the transformation efficiency?

A: Plating beads result in significantly higher transformation efficiency than using a plating loop.

Q: Do temperature and condensation of plating beads or plates affect transformation efficiency?

A: The transformation efficiency increases significantly when using dry plating beads and agar plates.

Q: What's the different of thawing the cells with circulating water instead of still water?

A: Transformation efficiency will increase 1.5 to 3-fold by thawing the cells with circulating water.

Q: What is the optimum incubation time on ice?

A: No significant difference between 1-10 minutes ice incubation. Over10 minutes, incubation will result in decreased efficiency.

Q: How do I prepare dry and warm selection plates?

A: After pouring plates, uncover the plates in a laminar flow, evaporate for 30-60 minutes. Then cover each plate and warm them at 37°C for more than 1 hour prior to transformation.

Q: Can HIT Competent Cells™ be freeze-thawed repeatedly?

A: Extensive freeze-thaw testing indicates HIT Competent Cells™ can be thawed, dispensed in aliquots and refrozen while maintaining 90%~100% efficiency if completed within 3 minutes. Use running water or water bath to rapidly thaw competent cells to about 1/3-volume thawed state (10-20 seconds). Incubate on ice until fully thawed (10-20 seconds) and immediately dispense on ice. Store cells at -70 °C. Maximum three times freeze thaw.

Q: What are the major differences between HIT Competent Cells™ strains?

A: HIT Competent Cells™ strains are common popular public lab strains. HIT Competent Cells™-DH5a is a strain which has been engineered for cloning large plasmids and library construction. HIT Competents Cells™-JM109 is a strain that grows faster and is excellent in blue/white and robotic screening. HIT Competent Cells™-Blue is also popular for regular cloning and blue/white screening. HIT Competent Cells™-21 is ideal for protein expression. More information could be found in application table and genotype table listed on page 3 and page 5.

Q: How do I reduce interference of satellite colonies?

A: 1. Using dry and warm plating beads and agar plates with proper antibiotics of suitable concentration.
2. Using fresh antibiotics.

Q: Does the concentration of ampicillin in the selection medium affect transformation efficiency?

A: For HIT Competent Cells™-DH5a: LB + Ap 50-60 μg/μl results in 2~3 times more transformation efficiency than LB + Ap 100 μg/μl. Transformed colonies can be observed after 11~16 hours cultivation, but after 18 hours satellite populations will appear around positive colonies.

For HIT Competent Cells™ -JM109: LB + Ap 50-100 μg/μl brings similar transformation efficiencies. Transformed colonies can be observed after 8~10 hours cultivation, but after 24 hours satellite colonies around positive colonies will also form.

Q: Does the size of plasmid affect transformation efficiency?

A: Refer to page 2, transformation efficiency = the numbers of transformed colonies/ mass of plasmids (μg). For instance, Super 10^9 competent cells can reach 1.6~5.5 x 10^9/μg with 2.7 kb plasmids, but only 4.0~9.0 x 10^6/ μg with 10 kb plasmids. The difference is about 100~1000 times.

Q: I can't see any colonies on my plate, is there something wrong with my HIT Competent Cells™?

A: It's highly unlikely to be a problem with HIT Competent Cells™ themselves since they are batch tested at manufacture and temperature controlled all the time during storage and shipping. Always use the control plasmid provided to perform a reference transformation experiment. If the control DNA is not transforming HIT Competent Cells™, are you sure the cells have been stored correctly? And have you followed the correct protocol? If yes, it's the time to contact your local distributor or Real Biotech Corporation.

HIT Competent Cells™

Ordering Information

HIT Competent Cells™

	Cat.No.	Items	Contents	Applications	Average Efficiency
DH5a	RH617	HIT™-DH5a Value 10^8	100 μl X 10 vials, 1 Control Plasmid	- blue/white screening, general cloning	2X10^8 transformants / μg pUC19
	RH618	HIT™-DH5a High 10^8	100 μl X 10 vials, 1 Control Plasmid	- blue/white screening for generation of cDNA libraries and subcloning	5X10^8 transformants / μg pUC19
	RH619	HIT™-DH5a Super 10^9	100 μl X 10 vials, 1 Control Plasmid	- blue/white screening for generation of cDNA libraries and subcloning	2X10^9 transformants / μg pUC19
	RH6110	HIT™-DH5a Bravo 10^9	100 μl X 10 vials, 1 Control Plasmid	- excellent for genomic and cDNA library construction and all cloning applications	8X10^9 transformants / μg pUC19
	RH617-J80	JUMBO HIT™-DH5a Value 10^8	100 μl X 80 vials, 1 Control Plasmid	- blue/white screening, general cloning	2X10^8 transformants / μg pUC19
	RH618-J80	JUMBO HIT™-DH5a High 10^8	100 μl X 80 vials, 1 Control Plasmid	- blue/white screening for generation of cDNA libraries and subcloning	5X10^8 transformants / μg pUC19
	RH619-J80	JUMBO HIT™-DH5a Super 10^9	100 μl X 80 vials, 1 Control Plasmid	- blue/white screening for generation of cDNA libraries and subcloning	2X10^9 transformants / μg pUC19
	RH6110-J80	JUMBO HIT™-DH5a Bravo 10^9	100 μl X 80 vials, 1 Control Plasmid	- excellent for genomic and cDNA library construction and all cloning applications	8X10^9 transformants / μg pUC19
	RH617-96	96-Well HIT™-DH5a Value 10^8	50 μl X 96-Well	- blue/white screening, general cloning	2X10^8 transformants / μg pUC19
	RH618-96	96-Well HIT™-DH5a High 10^8	50 μl X 96-Well	- blue/white screening for generation of cDNA libraries and subcloning	5X10^8 transformants / μg pUC19
	RH619-96	96-Well HIT™-DH5a Super 10^9	50 μl X 96-Well	- blue/white screening for generation of cDNA libraries and subcloning	2X10^9 transformants / μg pUC19
	RH6110-96	96 Well HIT™DH5a Bravo 10^9	50 μl X 96-Well	- excellent for genomic and cDNA library construction and all cloning applications	8X10^9 transformants / μg pUC19
GM2163	RH317	HIT™-GM2163 Value 10^8	100 μl X 10 vials, 1 Control Plasmid	- progation of plasmid free of Dam and Dcm methylations	1X10^8 transformants / μg pUC19
	RH317-J80	JUMBO HIT™-GM2163 Value 10^8	100 μl X 80vials, 1 Control Plasmid	- progation of plasmid free of Dam and Dcm methylations	1X10^8 transformants / μg pUC19
	RH317-96	96-Well HIT™ -GM2163 Value 10^8	50 μl X 96-Well	- progation of plasmid free of Dam and Dcm methylations	1X10^8 transformants / μg pUC19

Ordering Information

HIT Competent Cells™

	Cat.No.	Items	Contents	Applications	Average Efficiency
DH10B	RH517	HIT™-DH10B Value 10^8	100 µl X 10 vials, 1 Control Plasmid	- blue/white screening, general cloning	1X10^8 transformants / µg pUC19
	RH518	HIT™-DH10B High 10^8	100 µl X 10 vials, 1 Control Plasmid	- blue/white screening for generation of cDNA libraries and subcloning	2X10^8 transformants / µg pUC19
	RH517-J80	JUMBO HIT™-DH10B Value 10^8	100 µl X 80 vials, 1 Control Plasmid	- blue/white screening, general cloning	1X10^8 transformants / µg pUC19
	RH518-J80	JUMBO HIT™-DH10B High 10^8	100 µl X 80 vials, 1 Control Plasmid	- blue/white screening for generation of cDNA libraries and subcloning	2X10^8 transformants / µg pUC19
	RH517-96	96-Well HIT™-DH10B Value 10^8	50 µl X 96-Well	- blue/white screening, general cloning	1X10^8 transformants / µg pUC19
	RH518-96	96-Well HIT™-DH10B High 10^8	50 µl X 96-Well	- blue/white screening for generation of cDNA libraries and subcloning	2X10^8 transformants / µg pUC19
JM109	RH717	HIT™-JM109 Value 10^8	100 µl X 10 vials, 1 Control Plasmid	- 8-10 hours growth, blue / white screening, robotic screening, general cloning	1X10^8 transformants / µg pUC19
	RH718	HIT™-JM109 High 10^8	100 µl X 10 vials, 1 Control Plasmid	- 8-10 hours growth, blue / white screening, robotic screening, general cloning	3X10^8 transformants / µg pUC19
	RH717-J80	JUMBO HIT™-JM109 Value 10^8	100 µl X 80 vials, 1 Control Plasmid	- 8-10 hours growth, blue / white screening, robotic screening, general cloning	1X10^8 transformants / µg pUC19
	RH718-J80	JUMBO HIT™-JM109 High 10^8	100 µl X 80 vials, 1 Control Plasmid	- 8-10 hours growth, blue / white screening, robotic screening, general cloning	3X10^8 transformants / µg pUC19
	RH717-96	96-Well HIT™-JM109 Value 10^8	50 µl X 96-Well	- 8-10 hours growth, blue / white screening, robotic screening, general cloning	1X10^8 transformants / µg pUC19
	RH718-96	96-Well HIT™-JM109 High 10^8	50 µl X 96-Well	- 8-10 hours growth, blue / white screening, robotic screening, general cloning	3X10^8 transformants / µg pUC19

HIT Competent Cells™

Ordering Information

HIT Competent Cells™

	Cat.No.	Items	Contents	Applications	Average Efficiency
XL1-Blue	RH117	HIT™-Blue Value 10^8	100 µl X 10 vials, 1 Control Plasmid	- general cloning, blue / white screening, libraries	1X10^8 transformants / µg pUC19
	RH118	HIT™-Blue High 10^8	100 µl X 10 vials, 1 Control Plasmid	- general cloning, blue / white screening	5X10^8 transformants / µg pUC19
	RH119	HIT™-Blue Super 10^9	100 µl X 10 vials, 1 Control Plasmid	- general cloning, blue / white screening, libraries	2X10^9 transformants / µg pUC19
	RH117-J80	JUMBO HIT™-Blue Value 10^8	100 µl X 80 vials, 1 Control Plasmid	- general cloning, blue / white screening, libraries	1X10^8 transformants / µg pUC19
	RH118-J80	JUMBO HIT™-Blue High 10^8	100 µl X 80 vials, 1 Control Plasmid	- general cloning, blue / white screening	5X10^8 transformants / µg pUC19
	RH119-J80	JUMBO HIT™-Blue Super 10^9	100 µl X 80 vials, 1 Control Plasmid	- general cloning, blue / white screening, libraries	2X10^9 transformants / µg pUC19
	RH117-96	96-Well HIT™-Blue Value 10^8	50 µl X 96-Well	- general cloning, blue / white screening, libraries	1X10^8 transformants / µg pUC19
	RH118-96	96-Well HIT™-Blue High 10^8	50 µl X 96-Well	- general cloning, blue / white screening	5X10^8 transformants / µg pUC19
	RH119-96	96-Well HIT™-Blue Super 10^9	50 µl X 96-Well	- general cloning, blue / white screening, libraries	2X10^9 transformants / µg pUC19
BL21(DE3)	RH217	HIT™-21 Value 10^7	100 µl X 5 vials, 1 Control Plasmid	- general cloning protein expression	3X10^7 transformants / µg pUC19
	RH217-J40	JUMBO HIT™-21 Value 10^7	100 µl X 40 vials, 1 Control Plasmid	- general cloning protein expression	3X10^7 transformants / µg pUC19
	RH217-96	96-Well HIT™-21 Value 10^7	50 µl X 96-Well	- general cloning protein expression	3X10^7 transformants / µg pUC19
	RG001	RBC Glass Plating Beads, Sterile	100 g, 4 mm	- spread competent cells, 75~100 plates/bottle	