

10⁷ to 10⁹ Efficiency: High Ice Transformation



Real Biotech Corporation

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Non Heat Shock Transformation: Protocol Book

Product description

Cat. No.	Items	Strain	Spec	Notes
RH617-J	JUMBO HIT-DH5a	DH5a	1 mL X 2	- blue/white screening, general cloning - >5X10 ⁷ transformants/ μg pUC19
RH617	Value 10 ⁸ HIT-DH5a	DH5a	100 μL X 10	- blue/white screening, general cloning - >5X10 ⁷ transformants/ μg pUC19
RH 618	High 10 ⁸ HIT-DH5a	DH5a	100 μL X 10	- blue/white screening for generation of cDNA libraries and subcloning - >1X10 ⁸ transformants/ μg pUC19
RH619	Super 10 ⁹ HIT-DH5a	DH5a	100 μL X 10	- blue/white screening for generation of cDNA libraries and subcloning - >5X10 ⁸ transformants/ μg pUC19
RH717-J	JUMBO HIT-JM109	JM109	1 mL X 2	- 8-10 hours growth, blue/white screening, robotic screening, general cloning - >5X10 ⁷ transformants/ μg pUC19
RH717	Value 10 ⁸ HIT-JM109	JM109	100 μL X 10	- 8-10 hours growth, blue/white screening, robotic screening, general cloning - >5X10 ⁷ transformants/ μg pUC19
RH718	High 10 ⁸ HIT-JM109	JM109	100 μL X 10	- 8-10 hours growth, blue/white screening, robotic screening, general cloning - >1X10 ⁸ transformants/ μg pUC19
RH117-J	JUMBO HIT-Blue	XI-Blue	1 mL X 2	- general cloning, blue/white screening - >5X10 ⁷ transformants/ μg pUC19
RH118	High 10 ⁸ HIT-Blue	XI-Blue	100 μL X 10	- general cloning, blue/white screening, libraries - >1X10 ⁸ transformants/ μg pUC19
RH117	Value 10 ⁸ HIT-Blue	XI-Blue	100 μL X 10	- general cloning, blue/white screening - >5X10 ⁷ transformants/ μg pUC19
RH119	Super 10 ⁹ HIT-Blue	XI-Blue	100 μL X 10	- general cloning, blue/white screening, libraries - >5X10 ⁸ transformants/ μg pUC19
RH217	HIT-21	BI21 (DE3)	100 μL X 5	- general cloning protein expression - >5X10 ⁶ transformants/ μg pUC19
RG001 (autoclaved)	HIT plating beads		100g, 4mm	-spread competent cells, 75-100 plates

Competent cells must be stored in a stable -70C to -80C refrigerator

Fastest worldwide: 1 min protocol

RBC HIT *E. coli* competent cells provide the fastest true single-step transformation process world-wide (1 tube, from transformation to plating)

High efficiency:

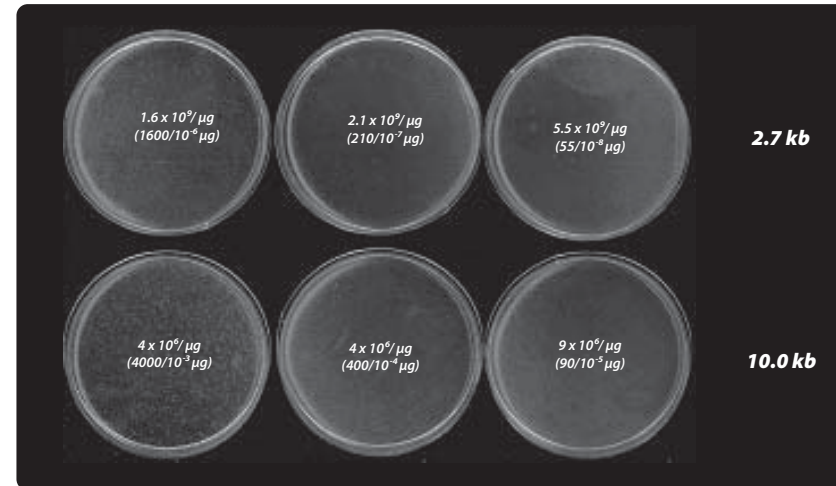
10^7 - 10^9 transformants/ μ g pUC19 plasmid

E. coli DNA transformation efficiency reaches 10^7 - 10^9 transformants/ μ g pUC19 plasmid DNA (varies according to strains and plasmid size)

Calculation of transformation efficiency

Method	HIT Non-Heat Shock Protocol
Formula	transformation efficiency = (transformed colonies) / (μ g of plasmid)
Example	$5.5 \times 10^9 / \mu$ g (efficiency) = 55 (transformed colonies) / $10^{-8} \mu$ g
Test for	RH619: Super 10^9 HIT-DH5a
Selection	LB agar (Ap 50 μ g/ml)
Results	test with 10^{-6} - $10^{-8} \mu$ g 2.7 kb plasmid, resulted in efficiency of 1.6 - $5.5 \times 10^9 / \mu$ g test with 10^{-3} - $10^{-5} \mu$ g 10.0 kb plasmid, resulted in efficiency of 4.0 - $9.0 \times 10^6 / \mu$ g

Plasmid size



Contents and Notes

- 1 JUMBO HIT: 10^{7-8} Efficiency (Requires Freeze-Thaw aliquoting)**
- 2 vials of 1 ml JUMBO HIT competent cells (should be stored at -70°C ~- 80°C)
- QC report and Control plasmid (pUC19, $10^{-4} \mu$ g/ μ l, stored at -20°C ~- 70°C)
- 2 Value 10^8 : 10^{7-8} Efficiency (Ready to Use)**
- 10 vials of 100 μ l Value 10^8 HIT competent cells (should be stored at -70°C ~- 80°C)
- QC report and Control plasmid (pUC19, $10^{-4} \mu$ g/ μ l, stored at -20°C ~- 70°C)
- 3 High 10^8 HIT: 10^8 Efficiency (Ready to Use)**
- 10 vials of 100 μ l High 10^8 HIT competent cells (should be stored at -70°C ~- 80°C)
- QC report and Control plasmid (pUC19, $10^{-4} \mu$ g/ μ l, stored at -20°C ~- 70°C)
- 4 Super 10^9 HIT: 10^9 Efficiency (Ready to Use)**
- 10 vials of 100 μ l Super 10^9 HIT competent cells (should be stored at -70°C ~- 8°C)
- QC report and Control plasmid (pUC19, $10^{-4} \mu$ g/ μ l, stored at -20°C ~- 70°C)

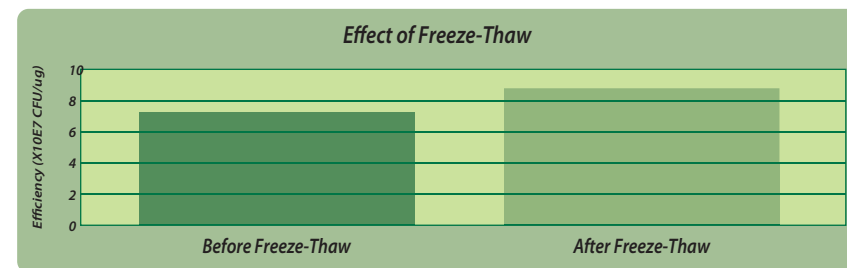
HIT QC Reports : Now Online!

Each batch of HIT competent cells is rigorously 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 cells for a complete report. Each HIT shipment is -70°C electronically monitored and recorded for minimum efficiency guarantee.

Expiry date: 1 year from date of manufacture

Notes for dispensing JUMBO HIT competent cells

JUMBO HIT may be dispensed in aliquots and refrozen with extremely high efficiency (90-100%). The whole procedure must be completed within 5 min. Use running tap water or water bath to fast thaw competent cells to 1/3-volume thawed state (10-20 sec.)-- incubate on ice until almost fully thawed (10-20 sec.)-- dispense into 100 ul aliquots on ice and immediately refrigerate at -70 ~- 80°C .



Genotypes of RBC HIT strains

Genotypes	Applications	HIT-DH5a	HIT-JM109	HIT-Blue	HIT- 21
		<i>F-</i> (80d lacZ M15) (lacZYA-argF)U169 hsdR17(r - m +) recA1 endA1 relA1 deoR	<i>F'</i> traD36 proA+ proB+ lacIq (lacZ)M15 I (lac-proAB) hsdR17 recA1 endA1 relA1	hsdR17(rk- mk+), recA1, endA1, gyrA96, thi-1, supE44, relA1, lac[F' proAB lacIqZDM15Tn10(Tet ^r)]	<i>E. coli</i> B, <i>F-</i> , <i>dcm</i> , <i>ompT</i> , <i>hsdS</i> (rB-mB-), <i>gal</i> (DE3)
<i>end A</i>	Prevents plasmid degradation during extraction	Yes	Yes	Yes	No
<i>recA</i>	Prevents DNA recombination	Yes	Yes	Yes	No
<i>hsd</i>	Enhances transformation efficiency of selected PCR DNA strands and cDNA libraries	Yes	Yes	Yes	Yes
<i>deoR</i>	Enhances transformation efficiency of high MW plasmids and cosmids	Yes	No	No	No
<i>LacZ M15</i>	Blue-White screening	Yes	Yes	Yes	No
<i>Lon</i>	Lon Protease Deficient, Improves Protein Yield	No	No	No	Yes
<i>ompT</i>	OmpT Protease Deficient, Improves Protein Yield	No	No	No	Yes

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HIT Non-Heat Shock Transformation Protocol

(1-10 minutes, efficiency = 10⁷~10⁹/μg)

Attention: Prior to transformation, dry plating beads and agar plates should be warmed to 37°C (strongly recommended)

PROTOCOL

Prepare ice bucket, 37°C plating beads and selective plates. Thaw competent cell vial with room-temp. tap water or water bath for 10~20 seconds until 1/3 thawed.

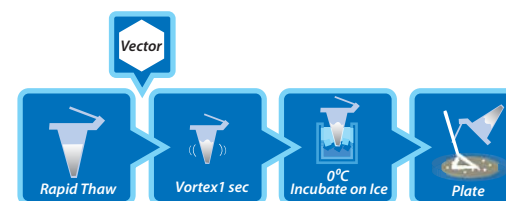
Add DNA whose volume is less than 10% of volume of cells . Vortex 1 second.

Place on ice for 1-10 minutes

Transfer onto 37°C dry selection plate media, spread using RBC plating beads.

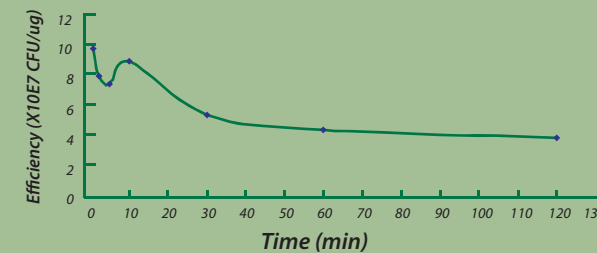
Immediately incubate plate at 37°C (16-18 hours for HIT-DH5a, 8-24 hours for HIT-109). Observe growth of transformed colonies.

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10⁷ to 10⁹ Efficiency: High Ice Transformation

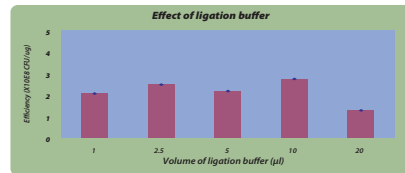
Time VS Efficiency



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Notes

1. RBC 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 cells can withstand high speed vortex)
3. Modified protocol for large plasmids (>6 kb) and cDNA libraries: 20-min ice bath followed by 1-min 42°C water bath and another 20-min ice bath. Efficiency will increase 2-5 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: 50µg/ml; Km: 25µg/ml, Tc: 12.5µg/ml for HIT-DH5a and HIT-JM109 strains. Higher antibiotic concentrations will decrease the efficiency. Lower conc. will increase the number of satellite colonies.
7. HIT-DH5a and HIT- JM109: Warning- over incubation at 37°C for 18-24 hours will result in satellite (pseudo-positive) colonies appearing.



Q & A

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 5 min. Use running water or water bath to rapidly thaw competent cells to about 1/3-volume thawed state (10-20 sec.). Incubate on ice until fully thawed (10-20 sec.) and immediately dispense on ice. Store cells at -70 °C. Maximum three times freeze thaw.

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

A: HIT competent cells should be stored at -70°C~-80°C condition. Slow thawing caused by power cuts and unstable freezers will result in decreased efficiency.

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 efficiencies than seen using a plating loop, probably due to increased surface spreading.

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

A: Use plating beads and plates at 37°C to greatly increase transformation efficiency. In addition, compared with wet plating beads and plates, dried plating beads and plates give increased transformation efficiency and lower numbers of satellite colonies.

Q: How to make dry and warm selection plates?

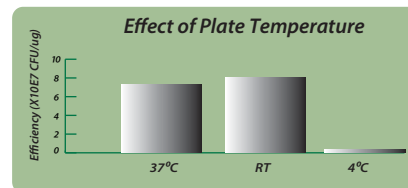
A: After pouring plates, uncover the plates in a laminar flow, evaporate for 30-60 min, cover each plate and warm in incubator prior to transformation at 37°C.

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 20min ice bath-1 min 42C Heat Shock - 20 min ice bath protocol to increase transformation efficiency.

Q: What is the optimum incubation time on ice?

A: Between 1-10 minute incubation will have no significant difference. Over ten minutes incubation will result in decreased efficiency, refer to Time VS Efficiency figure on pg. 10.



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

A: For HIT-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-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: As per the calculation shown on page 5, transformation efficiency = the numbers of transformed colonies/ mass of plasmids (µg). For instance, Super 10⁹ competent cells can reach 1.6~5.5 x 10⁹/µg with 2.7-kb plasmids, but only 4.0~9.0 x 10⁹/µg with 10.0-kb plasmids. The difference is about 100~1000 times.

Q: What are the major differences between HIT-DH5a, HIT-JM109, HIT-Blue and HIT-21?

A: Please refer to product table and genotype table above. More information in RBC Product Guide.

Q: How do I reduce interference of satellite colonies?

- A:**
1. Use warm and dry plating beads and plates.
 2. Please check and test the antibiotics, since the efficacy of antibiotics may be influenced by the method of preparation, different batches, sources and expiration dates.