Asia Hepato Gene Co.
Product Datasheet

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Competent cell preparation buffer

Application:
1. DH5α, JM109, HB101, BL21 Competent cell preparation
2. DH5α (BCRC Number: 51731) as standard, some differences between strains
3. Competent cell can be kept for at least two months

Kit Components:

Cat. No	Transform efficiency	Reaction	Components	Amount	Storage
CoCell-1	5×10^7~3×10^8 cfu/μg	500~100	Buffer A	2ml×50	25℃
			Control plasmid(3kbp)	0.1ng	-20℃
CoCell-2	5×10^7~3×10^8 cfu/μg	2600~520	Buffer A powder	8g×2	25℃
			Control plasmid(3kbp)	0.1ng	-20℃

Self prepare solution
DMSO (Dimethyl sulfoxide)
LB and SOC medium
0.5mg/ml Ampicillin
1×TE buffer: 10mM Tris-HCl pH8.0, 1mM EDTA

Buffer preparation:
Buffer A: Add 200ml ddH2O to dissolve 8g Buffer A powder (Cat. No. CoCell-2),
After then filtrate with 0.22μm sterile filter into a sterile bottle.
Control plasmid: add 1ml 1×TE buffer concentration 10^{-4}ng/μl, store at -20℃

Competent cell preparation:
*Make sure each step keep the cell in sterile environment
1-1. Transform efficiency ~3×10^8 cfu/μg , 2 vial
↓ Culture 1ml LB media DH5α (BCRC Number: 51731) in 15ml sterile centrifuge tube at 37℃, 120 rpm for 16~17 hours.
↓ Add 16μl overnight culture into 1.5 ml LB media in 15ml sterile centrifuge tube
↓ Culture at 37℃, 120 rpm for 2.5 hours (more or less time will affect transform efficiency)
↓ Pour 1.5ml DH5α into1.5ml sterile centrifuge tube
↓ Centrifuge 10,000 rpm for 1 min then remove LB
↓ Resuspend the pellet with 0.9ml Buffer A
↓ Stand for 20 minutes
↓ Centrifuge 10,000 rpm for 1 min then remove Buffer A
↓ Resuspend the pellet with 200μl Buffer A＋16μl DMSO
↓ Stand for 10 minutes
↓ Aliquot 2 vials (108μl/vial)
↓ Store at -70℃~-80℃ ready for use

1-2. Transform efficiency ~3×10^8 cfu/μg , 65 vial

↓ Culture 1ml LB media DH5α (BCRC Number: 51731) in 15ml sterile centrifuge tube at 37℃, 120 rpm for 16~17 hours.
↓ Add 0.5ml overnight culture into 50 ml LB media in 125ml sterile flask
↓ Culture at 37℃, 120 rpm for 2.5 hours (more or less time will affect transform efficiency)
↓ Pour 50ml DH5α into 50ml sterile centrifuge tube
↓ Centrifuge 5,000 rpm for 5 min then remove LB
↓ Resuspend the pellet with 28ml Buffer A
↓ Stand for 20 minutes
↓ Centrifuge 5,000 rpm for 5 min then remove Buffer A
↓ Resuspend the pellet with 6.5ml Buffer A＋520μl DMSO
↓ Stand for 10 minutes
↓ Aliquot 65 vials (108μl/vial)
↓ Store at -70℃~-80℃ ready for use

2. Transform efficiency ＞5×10^7 cfu/μg, 1×5 vial

5-fold dilute ~3×10^8 cfu/μg competent cell
↓ Mix 400μl Buffer A＋32μl DMSO into 1 vial (108μl) of ~3×10^8 cfu/μg competent cell
↓ Aliquot 5 vials, each vial 108μl
↓ Store at -70~-80℃ ready for use

Quality control:

	Transform efficiency	Contamination Test
1	Thaw 1 vial ~3×10^8 cfu/μg competent cell at room temperature before use	Thaw 1 vial ~3×10^8 cfu/μg competent cell at room temperature before use
2	Mix 1μl control plasmid (10^{-4} ng/μl) into competent cell	Mix 0μl control plasmid
3	4℃ for 5 minutes	4℃ for 5 minutes
4	42℃ 45 seconds	42℃ 45 seconds
5	Add 60μl SOC Medium	Add 60μl SOC Medium
6	Spread 168μl cells onto 20-50μg/ml Ampicillin plate. Excessive antibiotic significantly decreased the transformation efficiency	Spread 168μl cells onto 20-50μg/ml Ampicillin plate.
7	37℃, 12~16 hours, see 40~130 colony	37℃, 12~16 hours, should see 0 colony. But several colony contaminate won't affect target colony selection.

Medium Ingredient

LB		SOC			
Tryptone	10 g/L	Tryptone	20 g/L	MgCl2	0.95 g/L
Yeast extract	5 g/L	Yeast Extract	5 g/L	MgSO4	1.2 g/L
NaCl	10 g/L	NaCl	0.5 g/L	Glucose	3.6 g/L
		KCl	0.186 g/L		