Supplementary Materials

Efficient 1-hydroxy-2-butanone production from 1,2-butanediol by whole cells of engineered *E. coli*

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Table S1. The primers used in this study.

Primers	Sequences	Source
SSBDH-F	CAGCAAATGGGTCGCGGATCCATGTCGACAGGTTTGAACG	*Bam*HI
SSBDH-R	CTCGAGTGCGGCCGCAAGCTTTTAACGATAAACCAGCCCG	*Hin*dIII
mBDH-F	CAGCAAATGGGTCGCGGATCCATGCGTTTCGACAATAAAGT	*Bam*HI
mBDH-F	CTCGAGTGCGGCCGCAAGCTTTCAAACGATCTTCGGTTGAC	*Hin*dIII
RRBDH-F	CAGCAAATGGGTCGCGGATCCATGGTTAATTTCAAGGGGAA	*Bam*HI
RRBDH-R	CTCGAGTGCGGCCGCAAGCTTTTACTGCGGTGGACGCACCA	*Hin*dIII
GDH-F	CAGCAAATGGGTCGCGGATCCATGTTGAGAATCATCCAGTC	*Bam*HI
GDH-R	CTCGAGTGCGGCCGCAAGCTTTCAGCGTTGGTGTTGTTGCA	*Hin*dIII
CaADH:S199A-F	GTGTTGGAGCAAGACCTGTTTGTGTTG	
CaADH:S199A-R	ACAGGTCTCGTTCCAACACCGATAATT	
P1	CAGCAAATGGGTCGCGGATCCATGGTTAATTTCAAGGGGAA	*Bam*HI
P2	GGTTCCACCTCCTAAGAAAACGATCGCCGGGGTTACTGCGG TGGACGCACCA	RBS sequence
P3	CCCCGGCGATCGTTTTCTTAGGAGGTGGAACCATGAAAGTC ACAGTTGTTGG	RBS sequence
P4	TCGAGTGCGGCCGCAAGCTTTTAAGCGTTAACTGATTGGG	*Hin*dIII
P5	CAGCAAATGGGTCGCGGATCCATGGTTAATTTCAAGGGGAA	*Bam*HI
P6	GGTTCCACCTCCTAAGAAAACGATCGCCGGGGTTACTGCGG TGGACGCACCA	RBS sequence
P7	CCCCGGCGATCGTTTTCTTAGGAGGTGGAACCATGAAAGTC ACAGTTGTTGG	RBS sequence
P8	TAAGAAAACGATCGCCGGGGTTAAGCGTTAACTGATTGGG	RBS sequence
P9	CCCCGGCGATCGTTTTCTTAGGAGGTGGAACCATGTTAGAC CAGCAAACCAT	RBS sequence
P10	TCGAGTGCGGCCGCAAGCTTTTATTCAACCGCTTGAGCGT	*Hin*dIII
P11	CAGCAAATGGGTCGCGGATCCATGGTTAATTTCAAGGGGAA	*Bam*HI
P12	GGTTCCACCTCCTAAGAAAACGATCGCCGGGGTTACTGCGG TGGACGCACCA	RBS sequence
P13	CCCCGGCGATCGTTTTCTTAGGAGGTGGAACCATGAAAGTC ACAGTTGTTGG	RBS sequence
P14	TCGAGTGCGGCCGCAAGCTTTTAAGCGTTAACTGATTGGG	*Hin*dIII
P15	CAGCAAATGGGTCGCGGATCCATGGTTAATTTCAAGGGGAA	*Bam*HI
P16	GGTTCCACCTCCTAAGAAAACGATCGCCGGGGTTACTGCGG TGGACGCACCA	RBS sequence
P17	CCCCGGCGATCGTTTTCTTAGGAGGTGGAACCATGAAAGTC ACAGTTGTTGG	RBS sequence
P18	TAAGAAAACGATCGCCGGGGTTAAGCGTTAACTGATTGGG	RBS sequence
P19	CCCCGGCGATCGTTTTCTTAGGAGGTGGAACCATGTTAGAC CAGCAAACCAT	RBS sequence
P20	TCGAGTGCGGCCGCAAGCTTTTATTCAACCGCTTGAGCGT	*Hin*dIII

Table S2. Sequence information of the genes used in this study.

Gene name	Sequence
ssbdh	ATGTCGACAGGTTTGAACGGAAAGGTAGCCATTATCACTGGCGCTGCGCGTGG CATTGGCCGGGGGATCGCACTGCGCCTGGCACAAGAGGGGGTCAACCTTGCGC TCTTGGATTTATCCGCAGATCAGCTTGGTATAGTCAGGAAAGAGGTGGAGTCTT TCGGGGTAAAAGCGACAACCTACGTTGCCGATATCAGCAAGCGCGAGGAGGT GTATGCGGCCATCGAGCACGTTGTGAGTACGCTGGGTACCTTGGATGTGATGAT TAATAACGCCGGCATTTCGCAGGTAAAACCCATCGCAGACGTGGTGCCGGAAG ATCTTGAGAAGATCCTGAACATCAATATCGGGGGGGTGACCTGGGGGATCCAG GCCGCCGCGGCGAAATTTAAACAGCTCAACAAAAACGGCAAGATCATCAATG CTTGTTCCATCGCCGGTCACGAGGGTTTTGCATTGCTGGGCGTGTACTCCGCCAC CAAATTCGCCGTACGCGCATTGACCCAGGTTGCGGCCAAGGAATACGCCAGCG ACAACATTACCGTCAACGCCTATTGCCCTGGAGTGGTCGGCACCGATATGTGG GTGGAAATAGATCAGCGTTTCTCGGAGATTACCGGTGCGCCGAAAGGTGAAAC CTATAAGAAGTACGTTGATGGCATTGCCTTGGGGCGCGCACAAACCCCGGCTG ACGTCGCGGCACTTGTCGCTTTTCTTTCCAGCGACGACGCTGCCTATATCACCG GTCAGTCGATTTTGACCGACGGCGGGCTGGTTTATCGTTAA
mbdh	ATGCGTTTCGACAATAAAGTGGTAGTGATCACCGGCGCAGGCAATGGGATGGG GGAAGCGGCAGCACGCCGATTCTCCGCCGAGGGCGCCGTGGTGGTGCTGGCGG ACTGGGCGAAGGATGCGGTAGATGCCGTTGCCGCCTCGCTGCCGAAAGGCAGG GCGCTGGCGGTACACATCGACGTTTCCGACCCGGTTGCGGTTGAAAAAATGAT GAACGAGGTGGCGGCAAAACTGGGCCGCATCGACGTGTTGCTGAACAATGCCG GCGTGCACGTGGCGGGTACAGTGCTGGAAACCAGCGTGGCCGACTGGCGGCGC ATCGCTGGGGTGGATATCGACGGCGTAGTGTTCTGTTCCAAATTCGCCATGCCT TATTTGCTGAAAACCAAGGGCTGTATCGTTAACACCGCCTCGGTATCCGGCCTG GGTGGCGACTGGGGCGCGGCATATTACTGCGCGGCGAAAGGGGCGGTGGTCA ACCTGACGCGCGCCATGGCGCTGGATCACGGCGGTGACGGCGTGCGCGTGAAC TCGGTGTGCCCGAGCCTGGTGAAAACCAATATGACCAATGGTTGGCCGCAAGA GATCCGCGACAAGTTCAACGAGCGCATTGCGTTGGGGCGTGCGGCAGAGCCGG AAGAAGTGGCGGCGGTAATGGCGTTTCTGGCCAGCGACGACGCCAGCTTTATC AACGGCGCCAACATCCCGGTTGACGGCGGCGCGACCGCATCAGACGGTCAAC CGAAGATCGTTTGA
rrbdh	ATGGTTAATTTCAAGGGGAAGATGATGAAAGCAGCTCGTTGGTATCAGGCGCG CGACATCCGTATTGATGATATTGAAGAACCTCAGGTTTCCGCCGGTAAGGTGAA AATCAAGGTAGCCTGGACCGGAATTTGCGGCAGCGACCTGCACGAATACCTCG CCGGACCGATTTTTGCCCCGGTAGGCAAACCTCACAAGCTAAGCCATGACATC GCGCCTATCGTCATGGGCCATGAGTTTTCCGGTGAAGTGGTAGACGTGGGTCGC GGCGTGACGAAATTCAAAGCCGGTGACCGCGTGGTCGTAGAGCCGATCCTGGC TTGTCGTCAGTGCGAAGCCTGCCGCGAAGGCAAATATAACCTGTGCGCCGATC TGGGTTTCCACGGTCTGTCCGGCGGTGGCGGCGGCTTCTCCTCCTTTACCATGGT AGACGAGCATATGGTACACCGCATGCCCGATGCGCTCAGCTATGAGCAAGGTG CGTTGGTAGAACCTGCGGCGGTAGCCCTTCATGCGGTGCGGATGAGCAAATTG AAAGCCGGGGATAAAGCGGCCGTCTTCGGTGCCGGCCCCATCGGCCTGCTGGT GATTGAAGCACTGCGCGCGGCCGGTGCGGCAGAAATTTATGTGGTCGAGCTGT CACCTCAGCGGGCAGAAAAAGCGCGCGAACTGGGGGCGAAAGTGGTGATCGA CCCCAGCAAAGACGATGCCGTCGCCACAATACGCGAGCTGAGCGCGGGTGGC GTTGACGTAGCGTTTGAAGTGACCGGCGTGCCGGTGGTGCTAAAACAGTGCAT CGACAGCACTCGCTACGAAGGCGAGACGATTATCGTCTCCATCTGGGAAGGGG AAGCGGCATTCCATCCGAACAAAGTGGTGCTCAGCGAACGTTCAGTCAAAGGG ATTATCGCTTATCGCCATATTTTCCCGGCGGTGATGGATCTGATGACCCAGGGC TACTTCCAGGCCGACAAGCTGGTGACCAAACGTATCGAACTGGCTGATTTGGTG

	GAGCAGGGTTTTGAAGCGCTGGTGAAAGAAAAACAACAGGTTAAAATCCTGGT GCGTCCACCGCAGTAA
gdh	ATGTTGAGAATCATCCAGTCTCCAGGCAAGTACATTCAGGGCGCCAATGCGTTG GTCGCCGTGGGCCAATACGCTAAAGCGCTTGCCGACCATTATTTCGTGATTGCC GACGACTTCGTCATGAAGCTGGCGGGCGATACCCTGATGGGCAGCCTGCAACA GCACGGGGTGAAACATCACGCCTGCCTGTTCAACGGCGAATGCTGCCATAAGG AAATCGACCGGTTGGGGCGCGAACTGAAGGCCCATGGCTGTCGCGGCGTGATT GGCGTCGGCGGCGGCAAAACCCTCGATACCGCCAAAGCCATCGCGCACTACCA GCAATTGCCGGTCGTGTTAATTCCCACCATTGCTTCCACCGATGCGCCGACCAG CGCGCTGTCGGTGATCTACACCGAACAGGGCGAATTTGCCGAGTACCTGATCT ACCCGCGCAACCCGGATATGGTGGTGATGGACAGCGCGATTATCGCCAAGGCG CCTGTGCGCCTGTTGGTCGCGGGAATGGGCGATGCGCTTTCCACCTATTTTGAG GCGCAGGCCTGTTTCGACGCTCAGGCAACCAGCATGGCGGGCGGCAAATCGAC GCTGGCGGCGCTCAGCCTGGCGCGACTGTGCTATGAGACGCTGCTGGCAGAAG GCGTCAAAGCCAAACTGGCGGTTGAGGCCGGCGTGGTGACGGAAGCGGTCGA GCGCATTATCGAGGCCAATACCTACCTGAGCGGCATTGGTTTTGAAAGCAGCG GGCTGGCGGCGGCGCACGCTATTCACAACGGTTTCACCGTGCTGGAAGAGTGC CATCACCTGTACCACGGCGAGAAAGTGGCTTTCGGCACGCTAGCGCAACTGGT GCTGCAAAACAGCAGCATGGCGCAGATCGAAACGGTGCTGGATTTCTGCCACC GCATCGGCTTGCCTGTCACACTGGCCGAGATGGGCGTTAGCGGCGATGCGGCA GAGAAAATCATGGCGGTTGCCCAAGCCAGCTGCGCCGCCGGCGAAACCATCC ACAACATGCCGTTCAAGGTGACGCCGGCCAGCGTGCAGGCTGCAATCTTGACG GCCGATCGGCTGGGCAGTGCCTGGCTGCAACAACACCAACGCTGA
Cbadh	ATGAAAGCAGCATTATGGTATGCAAAGAAAGATGTTAGAGTAGAGGAAATTG AAGAACCTAAAGTTACAGTTAATGGTGTAAAGATTAAAGTAAAATGGTGTGGA ATATGTGGATCAGATTTACATGAATATTTAGGAGGACCTATATTTATACCAGTA GGACAACCTCACCCATTAAGTGGTACAACTGCTCCAGTAGTTCTAGGACATGA ATTTTCAGGAGATGTCGTTGAAGTTGGTCCTAATGTAAAGAATTTTAAACCAGG AGATAGAGTAATAGTTGAACCTATAGTTGCATGTGGAAAATGTCCAGCGTGCTT AGAAGGAAAATATAATTTATGTTCATCATTAGGTTTCCATGGACTTTGCGGAAG TGGTGGAGGACTTGCTGAATATACAGTTTTCCCAGAAGAATTCGTACATAAGAT TCCAGATGAAATGTCTTACGAACAAGCTGCTTTAGTTGAGCCAATGGCAGTAGC ATTACATTCAATTAGAATTGGTAATTTTAGAACAGGGGATACTGCGTTAGTACT AGGATCTGGTCCAATAGGACTAGCAACTATTGAGTGCTTAAAAGCAGCCGGTG CAAAATTAATAATAGTATTACAAAGAAAATCTATAAGACAAGAGTATGCTAAA AGAGCAGGGGCAGATGTAGTATTAGATCCTAATGAAGTAAATATAGCAGAGG AAGTTAAGAAGCTTACTAACGGATTAGGAGTTGACGTGGCGTTTGAAACTACA GGAGCTCAAATAGGTTTTGATACAGGTATAGATAGCTTAAAGTTTGAAGGAAC TTTAGTTGTAACTAGCATATGGGAAAATGATGTTAAATTTAATCCTAATGTATTA GTATTCACTGAGAAGAAAATCATTGGAACATTAGCATACAGACATGAATTCCC AGCAACTATGGCTCAAATGAAAGATGGAAGAATAAAAGCAGAAGGATATGTA ACAAAGAAAATACATTTAGATGATATAGTTGAAGAAGGGTTTGGAGCATTAAC AGGTCCAGAAAAGAAGAAACATGTTAAGATATTAGTATCTCCAGACAAAGAA CTTGTATCTAAATCTTAA
Caadh	ATGAAAGGTTTTGCAATGTTAGGTATTAACAAATTAGGATGGATTGAAAAGAA AAACCCAGTGCCAGGTCCTTATGATGCGATTGTACATCCTCTAGCTGTATCCCC ATGTACATCAGATATACATACGGTTTTTGAAGGAGCACTTGGTAATAGGGAAA ATATGATTTTAGGCCATGAAGCTGTAGGTGAAATAGCCGAAGTTGGCAGCGAA GTTAAAGATTTTAAAGTTGGCGATAGAGTTATCGTACCATGCACAACACCTGAC TGGAGATCTTTAGAAGTCCAAGCTGGTTTTCAGCAGCATTCAAACGGTATGCTT

	GCAGGATGGAAGTTTTCCAATTTTAAAGATGGTGTATTTGCAGATTACTTTCATG TAAACGATGCAGATATGAATCTTGCCATACTCCCAGATGAAATACCTTTAGAA AGTGCAGTTATGATGACAGACATGATGACTACTGGTTTTCATGGAGCAGAACTT GCAGACATAAAAATGGGCTCCAGCGTTGTAGTAATTGGTATAGGAGCTGTTGG ATTAATGGGAATAGCCGGTTCCAAACTTCGAGGAGCAGGCAGAATTATCGGTG TTGGAAGCAGACCTGTTTGTGTTGAAACAGCTAAATTTTATGGAGCAACTGATA TTGTAAATTATAAAAATGGTGATATAGTTGAACAAATCATGGACTTAACTCATG GTAAAGGTGTAGACCGTGTAATCATGGCAGGCGGTGGTGCTGAAACACTAGCA CAAGCAGTAACTATGGTTAAACCTGGCGGCGTAATTTCTAACATCAACTACCAT GGAAGCGGTGATACTTTACCAATACCTCGTGTTCAATGGGGCTGCGGCATGGCT CACAAAACTATAAGAGGAGGATTATGCCCCGGCGGACGTCTTAGAATGGAAAT GCTAAGAGATCTTGTTCTATATAAACGTGTTGATTTGAGTAAACTTGTTACTCAT GTATTTGATGGTGCAGAAAATATTGAAAAGGCCCTTTTGCTTATGAAAAATAAG CCAAAAGATTTAATTAAATCAGTAGTTACATTCTAA
Caadh:S199A	ATGAAAGGTTTTGCAATGTTAGGTATTAACAAATTAGGATGGATTGAAAAGAA AAACCCAGTGCCAGGTCCTTATGATGCGATTGTACATCCTCTAGCTGTATCCCC ATGTACATCAGATATACATACGGTTTTTGAAGGAGCACTTGGTAATAGGGAAA ATATGATTTTAGGCCATGAAGCTGTAGGTGAAATAGCCGAAGTTGGCAGCGAA GTTAAAGATTTTAAAGTTGGCGATAGAGTTATCGTACCATGCACAACACCTGAC TGGAGATCTTTAGAAGTCCAAGCTGGTTTTCAGCAGCATTCAAACGGTATGCTT GCAGGATGGAAGTTTTCCAATTTTAAAGATGGTGTATTTGCAGATTACTTTCATG TAAACGATGCAGATATGAATCTTGCCATACTCCCAGATGAAATACCTTTAGAA AGTGCAGTTATGATGACAGACATGATGACTACTGGTTTTCATGGAGCAGAACTT GCAGACATAAAAATGGGCTCCAGCGTTGTAGTAATTGGTATAGGAGCTGTTGG ATTAATGGGAATAGCCGGTTCCAAACTTCGAGGAGCAGGCAGAATTATCGGTG TTGGAGCCAGACCTGTTTGTGTTGAAACAGCTAAATTTTATGGAGCAACTGATA TTGTAAATTATAAAAATGGTGATATAGTTGAACAAATCATGGACTTAACTCATG GTAAAGGTGTAGACCGTGTAATCATGGCAGGCGGTGGTGCTGAAACACTAGCA CAAGCAGTAACTATGGTTAAACCTGGCGGCGTAATTTCTAACATCAACTACCAT GGAAGCGGTGATACTTTACCAATACCTCGTGTTCAATGGGGCTGCGGCATGGCT CACAAAACTATAAGAGGAGGATTATGCCCCGGCGGACGTCTTAGAATGGAAAT GCTAAGAGATCTTGTTCTATATAAACGTGTTGATTTGAGTAAACTTGTTACTCAT GTATTTGATGGTGCAGAAAATATTGAAAAGGCCCTTTTGCTTATGAAAAATAAG CCAAAAGATTTAATTAAATCAGTAGTTACATTCTAA

Table 3S. Kinetic parameters of (2*R*, 3*R*)-BDH and (2*S*, 3*S*)-BDH for 1,2-BD mixture.

Kinetic parameters	(2*R*, 3*R*)-BDH	(2*S*, 3*S*)-BDH
V_{max} (U/mg)	23.18 ± 0.17	1.40 ± 0.03
K_m (mM)	0.97 ± 0.07	2.60 ± 0.32
K_{cat} (S^{-1})	16.61 ± 2.31	0.70 ± 0.12
K_{cat}/K_m (S^{-1} mM^{-1})	17.07 ± 1.34	0.27 ± 0.01

Table S4. Enzyme activities of the recombinant *E. coli* strains.

***E. coli* strains**	**Enzyme activities of (2*S*, 3*S*)-BDH, (2*R*, 3*R*)-BDH and NOX (U/mg)**	
	1,2-BD mixture	NADH
pET28a	0	ND
pET-*ssbdh*	0.36 ± 0.01	ND
pET-*ssbdh-nox*	0.25 ± 0.03	2.02 ± 0.23
pET-*ssbdh-nox-vgb*	0.23 ± 0.01	2.60 ± 0.16
pET-*rrbdh*	9.89 ± 0.31	ND
pET-*rrbdh-nox*	9.02 ± 0.47	2.49 ± 0.16
pET-*rrbdh-nox-vgb*	9.07 ± 0.24	2.58 ± 0.27

ND: not detected.

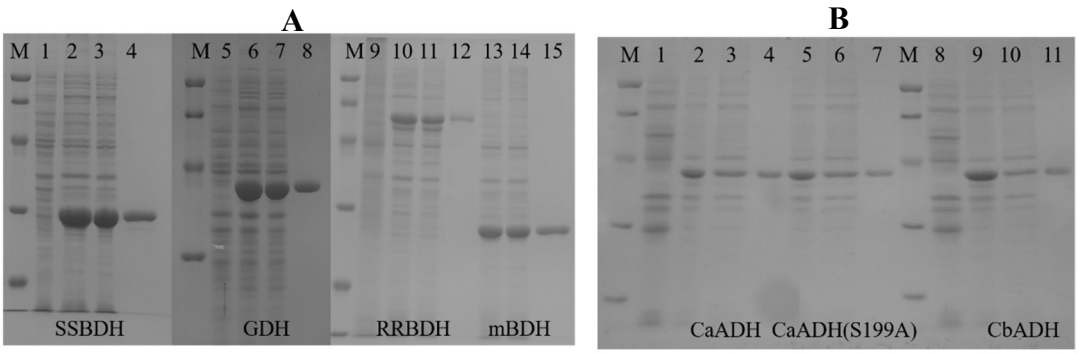

Figure S1. Analysis of expression and purification for SSBDH, GDH, RRBDH, mBDH (A), and CaADH, CaADH:S199A, CbADH (B) by SDS-PAGE. (A) Lane M, marker (97.2, 66.4, 44.3, 29, 20.01, 14.3 kDa); Lane 1-4, SSBDH (control, whole cell, soluble protein, and purified enzyme); Lane 5-8, GDH (whole cell, soluble protein, and purified enzyme); Lane 9-12, RRBDH (control, whole cell, soluble protein, and purified enzyme); Lane 13-15, mBDH (whole cell, soluble protein, and purified enzyme); (B) Lane M, marker (97.2, 66.4, 44.3, 29, 20.01, 14.3 kDa); Lane 1-4, CaADH, (control, whole cell, soluble protein, and purified enzyme); Lane 5-7, CaADH:S199A, (whole cell, soluble protein, and purified enzyme); Lane 8-11, CbADH (control, whole cell, soluble protein, and purified enzyme).

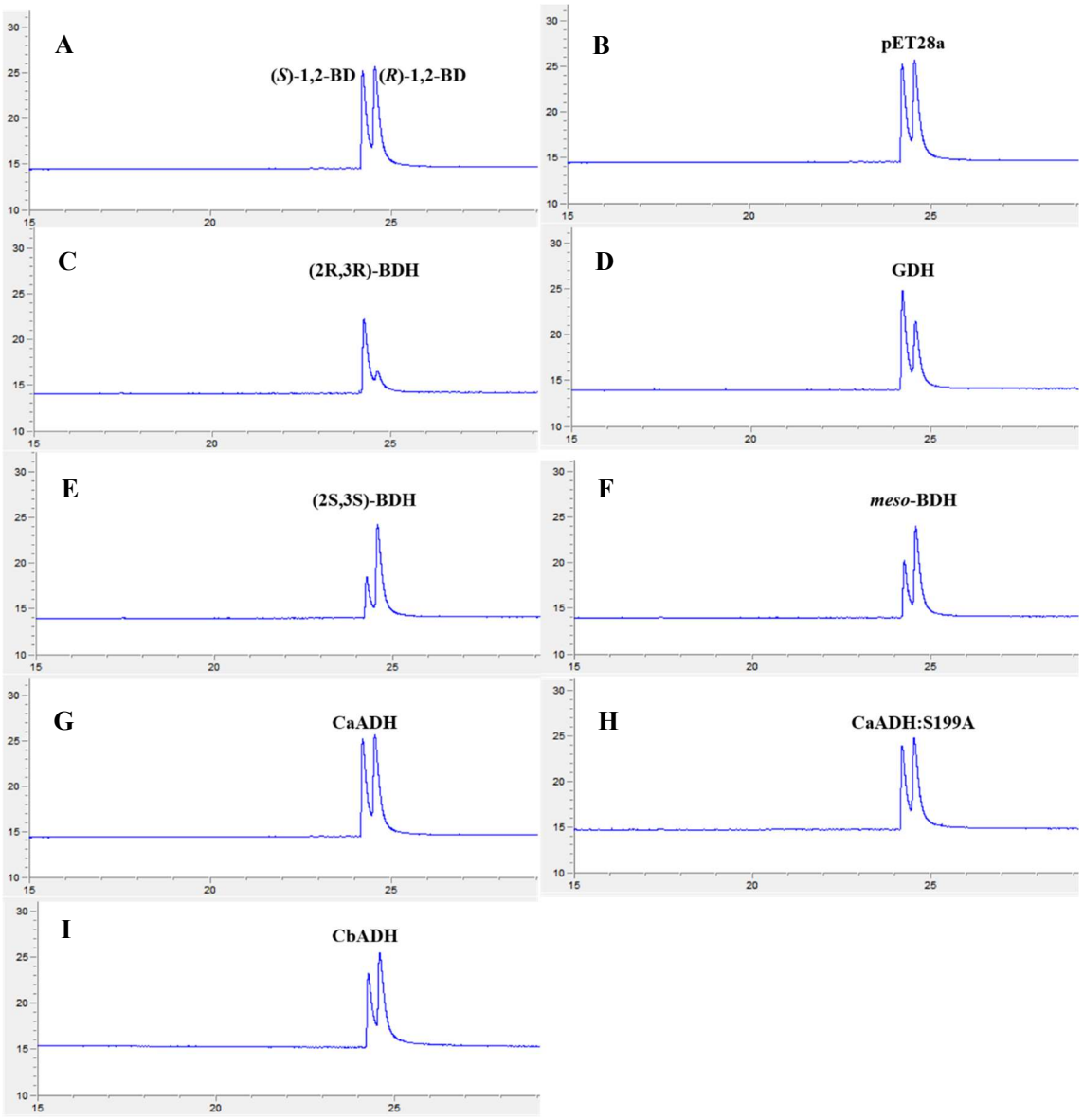

Figure S2. Chiral analysis of the product in the bioconversion reactions by whole cell biocatalysis. (A) Standard chemical; (B) *E. coli* (pET28a); (C) *E. coli* (pET-*rrbdh*); (D) *E. coli* (pET-*gdh*); (E) *E. coli* (pET-*ssbdh*); (F) *E. coli* (pET-*mbdh*); (G) *E. coli* (pET-*Caadh*); (H) *E. coli* (pET-*Caadh*:S199A); (I) *E. coli* (pET-*Cbadh*).

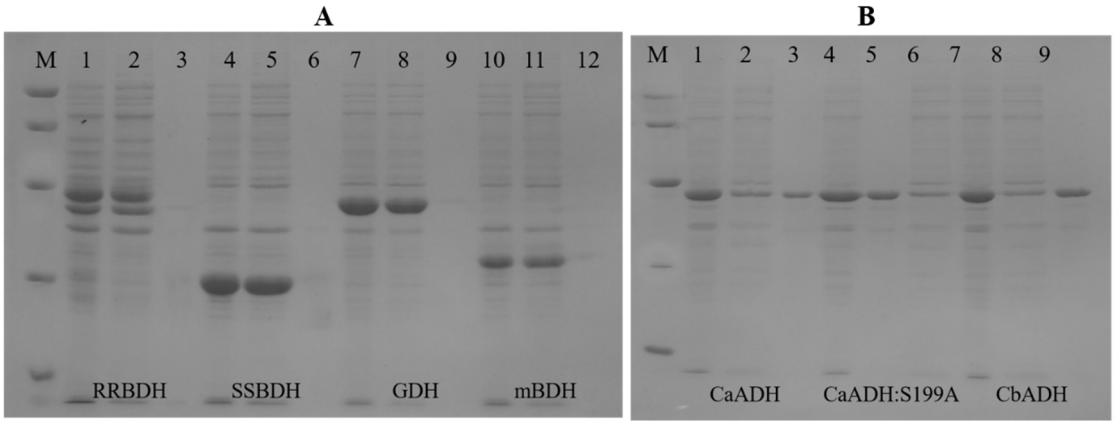

Figure S3. Analysis of expression level for RRBDH, SSBDH, GDH, mBDH, CaADH, CaADH:S199A, and CbADH in *E. coli* by SDS-PAGE. A: Lane M, marker (97.2, 66.4, 44.3, 29, 20.01, 14.3 kDa); Lane 1-3, RRBDH (whole cell, soluble protein, insoluble protein); Lane 4-6, SSBDH (whole cell, soluble protein, insoluble protein); Lane 7-9, GDH (whole cell, soluble protein, insoluble protein); Lane 10-12, mBDH, (whole cell, soluble protein, insoluble protein); B: Lane M, marker (97.2, 66.4, 44.3, 29, 20.01, 14.3 kDa); Lane 1-3, CaADH (whole cell, soluble protein, insoluble protein); Lane 4-6, CaADH:S199A (whole cell, soluble protein, insoluble protein); Lane 7-9, CbADH (whole cell, soluble protein, insoluble protein).

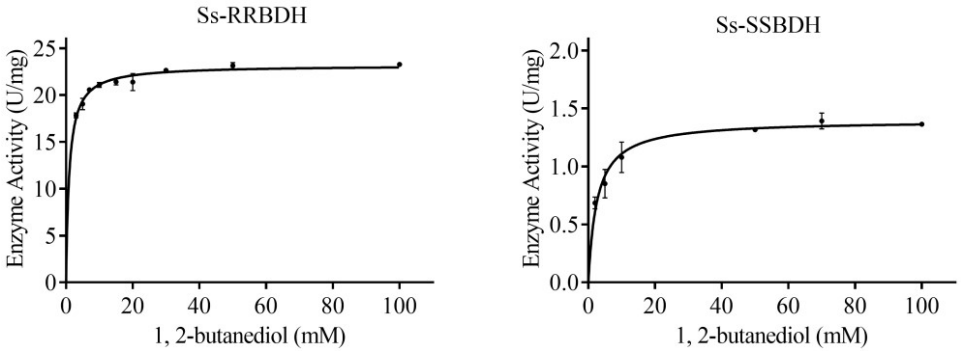

Figure 4S. Analysis of kinetic parameters for (2*R*, 3*R*)-BDH and (2*S*, 3*S*)-BDH using 1,2-BD mixture as substrate.

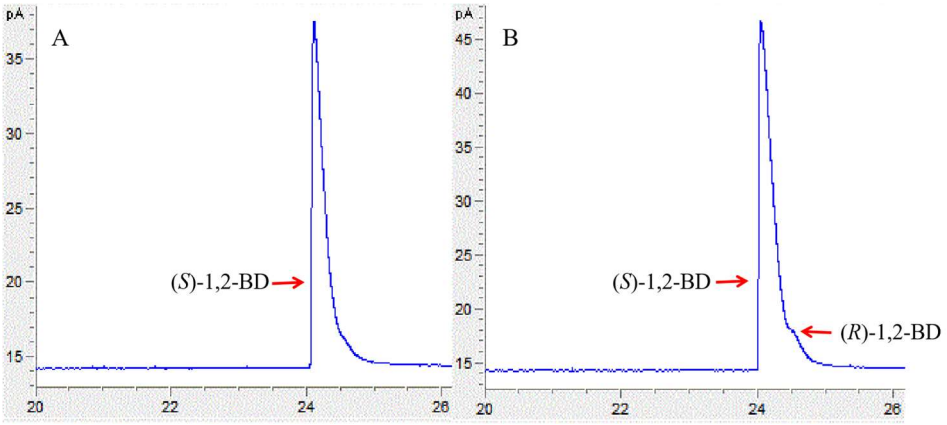

Figure S5. Chiral resolution of (*S*)-1,2-BD from 300 mM (A) and 400 mM (B) 1,2-BD mixture by *E. coli* (pET-*rrbdh-nox-vgb*).

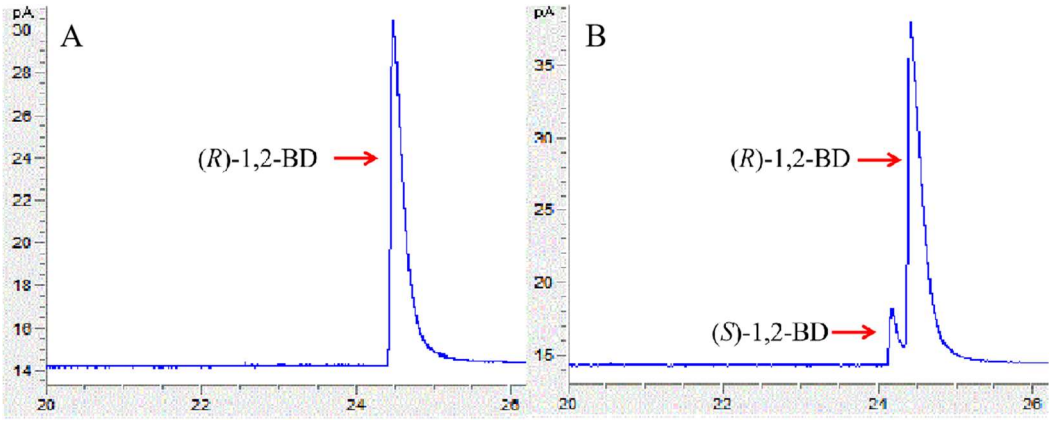

Figure S6. Chiral resolution of (*R*)-1,2-BD from 200 mM (A) and 300 mM (B) 1,2-BD mixture by *E. coli* (pET-*ssbdh-nox-vgb*).