

CX2-150USP1_ST25
SEQUENCE LISTING

<110> CODEXIS, INC.
OSBORNE, Robert
MITCHELL, Vesna
HTWE, Khin Yu Naing
ZHANG, Xiyun
MILCZEK, Erika M.
MOORE, Jeffrey C.

<120> NOVEL P450-BM3 VARIANTS WITH IMPROVED ACTIVITY

<130> CX2-150USP1

<160> 16

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<211> 3165

<212> DNA

<213> Artificial Sequence

<220>

<223> P450_BM3

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CX2-150USP1_ST25

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<211> 1054

<212> PRT

<213> Artificial Sequence

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<223> P450_BM3

<400> 2

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Leu Trp Arg Ile Ala Asp Glu Leu Gly Pro Ile Phe Arg Phe Asp Phe
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Pro Gly Val Ser Ser Val Phe Val Ser Gly His Asn Leu Val Ala Glu

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Lys Val Arg Ala Phe Ser Gly Asp Gly Leu Ala Thr Ser Trp Thr His					
	85	90	95		
Glu Pro Asn Trp Arg Lys Ala His Asn Ile Leu Met Pro Thr Phe Ser					
	100	105	110		
Gln Arg Ala Met Lys Asp Tyr His Glu Lys Met Val Asp Ile Ala Thr					
	115	120	125		
Gln Leu Ile Gln Lys Trp Ser Arg Leu Asn Pro Asn Glu Glu Ile Asp					
	130	135	140		
Val Ala Asp Asp Met Thr Arg Leu Thr Leu Asp Thr Ile Gly Leu Cys					
	145	150	155	160	
Gly Phe Asn Tyr Arg Phe Asn Ser Phe Tyr Arg Asp Gln Pro His Pro					
	165	170	175		
Phe Ile Thr Ser Met Val Arg Ala Leu Asp Glu Ala Met Asn Lys Leu					
	180	185	190		
Gln Arg Ala Asn Pro Asp Asp Pro Ala Tyr Asp Glu Asn Lys Arg Gln					
	195	200	205		
Phe Gln Glu Asp Ile Lys Val Met Asn Asp Leu Val Asp Ser Ile Ile					
	210	215	220		
Ala Glu Arg Arg Ala Asn Gly Asp Gln Asp Glu Lys Asp Leu Leu Ala					
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Arg Met Leu Asn Val Glu Asp Pro Glu Thr Gly Glu Lys Leu Asp Asp					
	245	250	255		
Glu Asn Ile Arg Phe Gln Ile Ile Thr Phe Leu Ile Ala Gly His Glu					
	260	265	270		
Thr Thr Ser Gly Leu Leu Ser Phe Ala Ile Tyr Cys Leu Leu Thr His					
	275	280	285		
Pro Glu Lys Leu Lys Lys Ala Gln Glu Glu Ala Asp Arg Val Leu Thr					
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Asp Asp Thr Pro Glu Tyr Lys Gln Ile Gln Gln Leu Lys Tyr Ile Arg					
	305	310	315	320	
Met Val Leu Asn Glu Thr Leu Arg Leu Tyr Pro Thr Ala Pro Ala Phe					
	325	330	335		
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	340	345	350		
Ser Lys Gly Gln Pro Val Thr Val Leu Ile Pro Lys Leu His Arg Asp					
	355	360	365		
Gln Asn Ala Trp Gly Pro Asp Ala Glu Asp Phe Arg Pro Glu Arg Phe					
	370	375	380		
Glu Asp Pro Ser Ser Ile Pro His His Ala Tyr Lys Pro Phe Gly Asn					
	385	390	395	400	
Gly Gln Arg Ala Cys Ile Gly Met Gln Phe Ala Leu Gln Glu Ala Thr					
	405	410	415		
Met Val Leu Gly Leu Val Leu Lys His Phe Glu Leu Ile Asn His Thr					
	420	425	430		
Gly Tyr Glu Leu Lys Ile Lys Glu Ala Leu Thr Ile Lys Pro Asp Asp					

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Phe	Lys	Ile	Thr	Val	Lys	Pro	Arg	Lys	Thr	Ala	Ala	Ile	Asn	Val	Gln
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Glu	Met	Lys	Phe	Ser	Glu	Phe	Ile	Ala	Leu	Leu	Pro	Ser	Ile	Arg	Pro

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Ser Ile Thr Val Ser Val Val Ser	Gly Glu Ala Trp Ser Gly Tyr Gly	
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865	870	875
Asp Thr Ile Thr Cys Phe Ile Ser Thr	Pro Gln Ser Glu Phe Thr Leu	
885	890	895
Pro Lys Asp Pro Glu Thr Pro Leu Ile	Met Val Gly Pro Gly Thr Gly	
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Val Ala Pro Phe Arg Gly Phe Val Gln	Ala Arg Lys Gln Leu Lys Glu	
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Gln Gly Gln Ser Leu Gly Glu Ala His	Leu Tyr Phe Gly Cys Arg Ser	
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Pro His Glu Asp Tyr Leu Tyr Gln Glu	Glu Leu Glu Asn Ala Gln Ser	
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Glu Gly Ile Ile Thr Leu His Thr Ala	Phe Ser Arg Met Pro Asn Gln	
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Pro Lys Thr Tyr Val Gln His Val Met	Glu Gln Asp Gly Lys Lys Leu	
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Ile Glu Leu Leu Asp Gln Gly Ala His	Phe Tyr Ile Cys Gly Asp Gly	
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Ser Gln Met Ala Pro Ala Val Glu Ala	Thr Leu Met Lys Ser Tyr Ala	
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Asp Val His Gln Val Ser Glu Ala Asp	Ala Arg Leu Trp Leu Gln Gln	
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<211> 3150

<212> DNA

<213> Artificial Sequence

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<223> P450_BM3

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CX2-150USP1_ST25

<211> 1049

<212> PRT

<213> Artificial Sequence

<220>

<223> P450_BM3

<400> 4

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35     40     45
Val Thr Arg Tyr Leu Ser Ser Gln Arg Leu Ile Lys Glu Ala Cys Asp
50     55     60
Glu Ser Arg Phe Asp Lys Asn Leu Ser Gln Ala Leu Lys Phe Ala Arg
65     70     75     80
Asp Phe Ala Gly Asp Gly Leu Val Thr Ser Trp Thr His Glu Ile Asn
85     90     95
Trp Lys Lys Ala His Asn Ile Leu Leu Pro Ser Phe Ser Gln Gln Ala
100    105    110
Met Lys Gly Tyr His Ala Met Met Val Asp Ile Ala Val Gln Leu Val
115    120    125
Gln Lys Trp Glu Arg Leu Asn Ala Asp Glu His Ile Glu Val Ser Glu
130    135    140
Asp Met Thr Arg Leu Thr Leu Asp Thr Ile Gly Leu Cys Gly Phe Asn
145    150    155    160
Tyr Arg Phe Asn Ser Phe Tyr Arg Asp Gln Pro His Pro Phe Ile Ile
165    170    175
Ser Met Val Arg Ala Leu Asp Glu Val Met Asn Lys Leu Gln Arg Ala
180    185    190
Asn Pro Asp Asp Pro Ala Tyr Asp Glu Asn Lys Arg Gln Cys Gln Glu
195    200    205
Asp Ile Lys Val Met Asn Asp Leu Val Asp Lys Ile Ile Ala Asp Arg
210    215    220
Lys Ala Arg Gly Glu Gln Ser Asp Asp Leu Leu Thr Gln Met Leu Asn
225    230    235    240
Gly Lys Asp Pro Glu Thr Gly Glu Pro Leu Asp Asp Gly Asn Ile Ser
245    250    255
Tyr Gln Ile Ile Thr Phe Leu Ile Ala Gly His Glu Thr Thr Ser Gly
260    265    270
Leu Leu Ser Phe Ala Leu Tyr Phe Leu Val Lys Asn Pro His Val Leu
275    280    285
Gln Lys Val Ala Glu Glu Ala Ala Arg Val Leu Val Asp Pro Val Pro
290    295    300
Ser Tyr Lys Gln Val Lys Gln Leu Lys Tyr Val Gly Met Val Leu Asn
305    310    315    320

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Glu	Val	Met	Val	Leu	Ile	Pro	Gln	Leu	His	Arg	Asp	Lys	Thr	Ile	Trp	355	360	365
Gly	Asp	Asp	Val	Glu	Glu	Phe	Arg	Pro	Glu	Arg	Phe	Glu	Asn	Pro	Ser	370	375	380
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Cys	Ile	Gly	Gln	Gln	Phe	Ala	Leu	His	Glu	Ala	Thr	Leu	Val	Leu	Gly	405	410	415
Met	Met	Leu	Lys	His	Phe	Asp	Phe	Glu	Asp	His	Thr	Asn	Tyr	Glu	Leu	420	425	430
Asp	Ile	Lys	Glu	Thr	Leu	Thr	Leu	Lys	Pro	Glu	Gly	Phe	Val	Val	Lys	435	440	445
Ala	Lys	Ser	Lys	Lys	Ile	Pro	Leu	Gly	Gly	Ile	Pro	Ser	Pro	Ser	Thr	450	455	460
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Lys	Gly	Val	Arg	Tyr	Ser	Val	Phe	Gly	Cys	Gly	Asp	Lys	Asn	Trp	Ala	565	570	575
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CX2-150USP1_ST25

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<223> P450_BM3

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CX2-150USP1_ST25

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<211> 1049

<212> PRT

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<223> P450_BM3

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CX2-150USP1_ST25

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CX2-150USP1_ST25

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CX2-150USP1_ST25

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CX2-150USP1_ST25

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<212> PRT

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<223> P450_BM3

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Ile Ala Asp Glu Leu Gly Glu Ile Phe Lys Phe Glu Ala Pro Gly Leu
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Val Thr Arg Tyr Leu Ser Ser Gln Arg Leu Ile Lys Glu Ala Cys Asp
          50          55          60
Glu Ser Arg Phe Asp Lys Asn Leu Ser Gln Ala Leu Lys Phe Ala Arg
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Asp Phe Ala Gly Asp Gly Leu Phe Thr Ser Trp Thr His Glu Lys Asn
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Tyr	Arg	Phe	Asn	Ser	Phe	Tyr	Arg	Asp	Gln	Pro	His	Pro	Phe	Ile	Thr		
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Ser	Met	Val	Arg	Ala	Leu	Asp	Glu	Ala	Met	Asn	Lys	Gln	Gln	Arg	Ala		
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Asn	Pro	Asp	Asp	Pro	Ala	Tyr	Asp	Glu	Asn	Lys	Arg	Gln	Phe	Gln	Glu		
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Tyr	Gln	Ile	Ile	Thr	Phe	Leu	Ile	Ala	Gly	His	Glu	Thr	Thr	Ser	Gly		
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Cys	Ile	Gly	Gln	Gln	Phe	Ala	Leu	His	Glu	Ala	Thr	Leu	Val	Leu	Gly		
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Thr	Pro	Leu	Leu	Val	Leu	Tyr	Gly	Ser	Asn	Met	Gly	Thr	Ala	Glu	Gly		

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CX2-150USP1_ST25

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CX2-150USP1_ST25

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Glu Ser Arg Phe Asp Lys Asn Leu Ser Gln Ala Leu Lys Phe Ala Arg
65     70     75     80
Asp Phe Leu Gly Asp Gly Leu Val Thr Ser Trp Thr His Glu Ile Asn
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Trp Lys Lys Ala His Asn Ile Leu Leu Pro Ser Phe Ser Gln Gln Ala
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Asn Pro Asp Asp Pro Ala Tyr Asp Glu Asn Lys Arg Gln Cys Gln Glu
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Asp Ile Lys Val Met Asn Asp Leu Val Asp Lys Ile Ile Ala Asp Arg
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