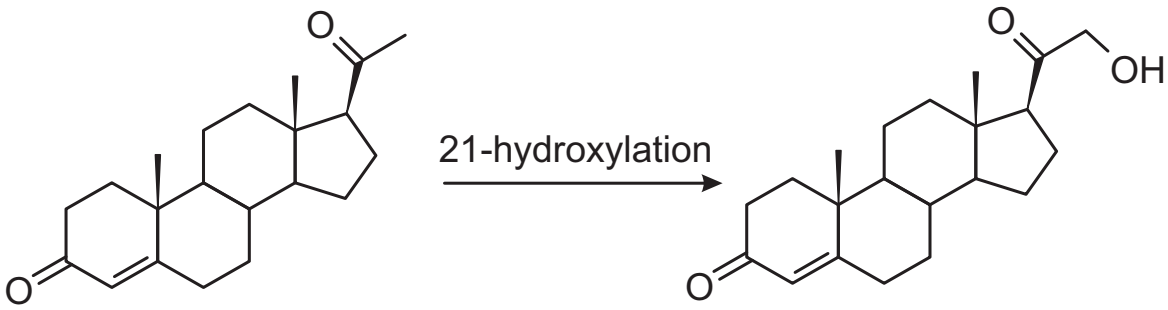
Figure 1



Progesteron

21-OH Progesteron

Figure 2

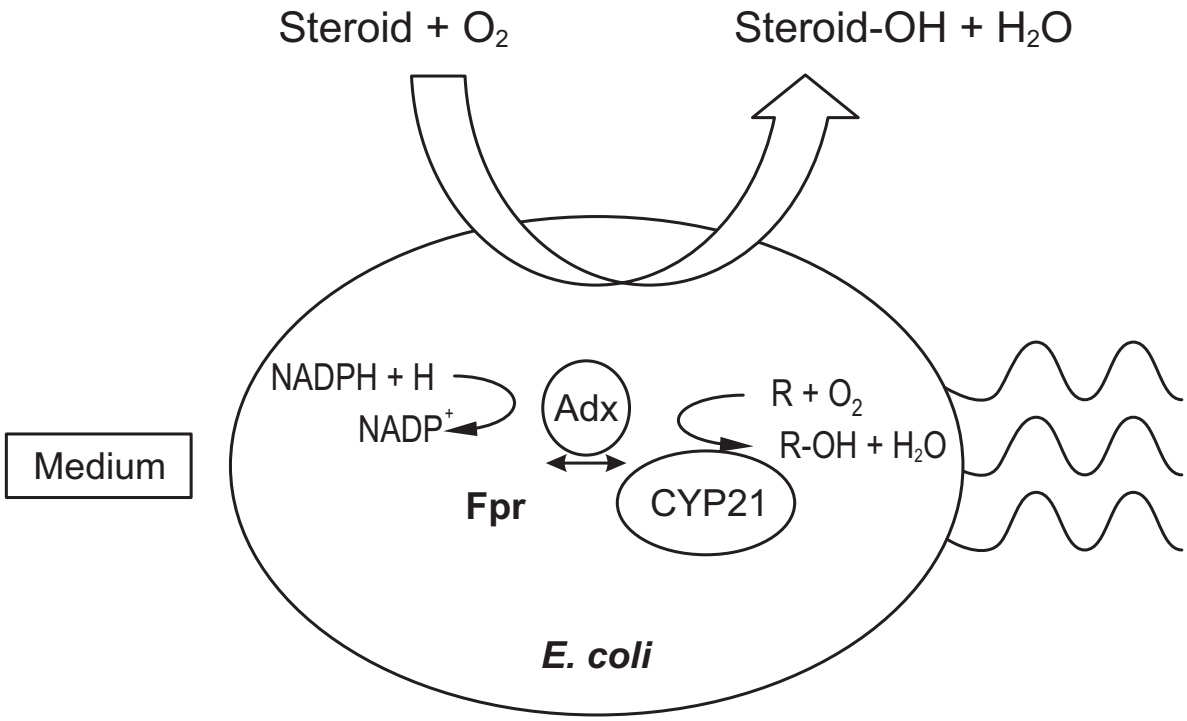
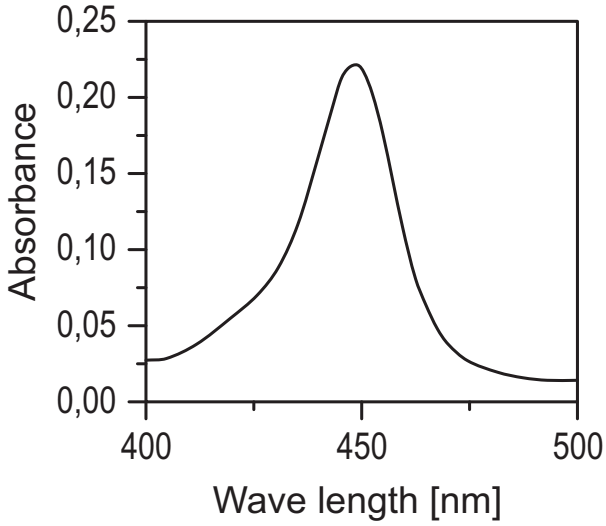

Steroid + O_2
Steroid-OH + H_2O
NADPH + H
$NADP^+$
Adx
R + O_2
R-OH + H_2O
Medium
Fpr
CYP21
E. coli

Figure 3


0,25
0,20
0,15
0,10
0,05
0,00
Absorbance
400
450
500
Wave length [nm]

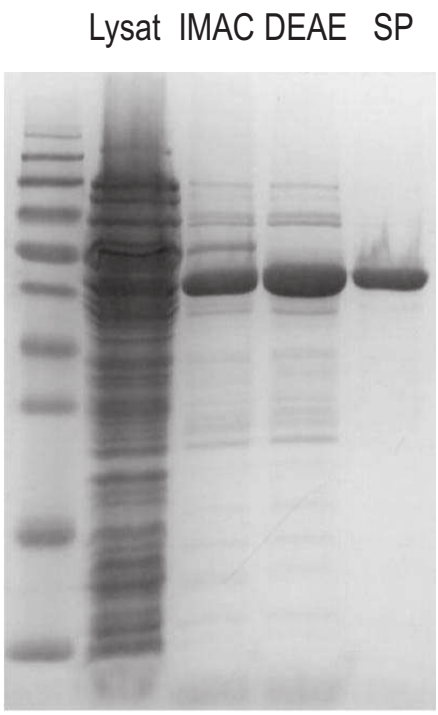

Lysat IMAC DEAE SP

Figure 4

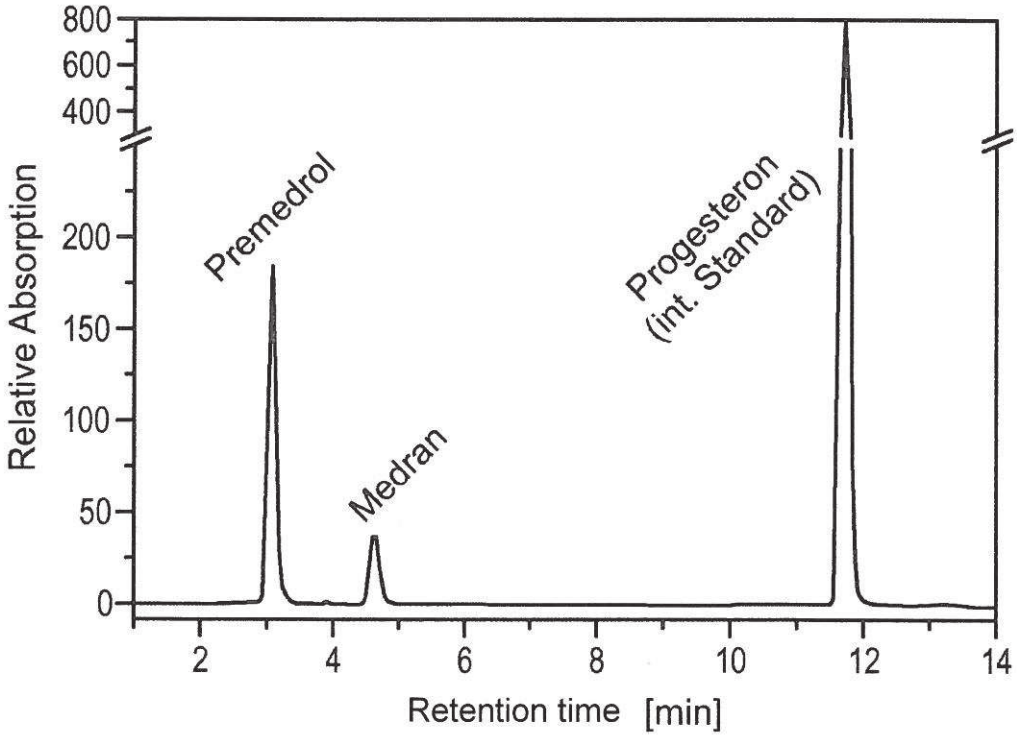

800
600
400
200
150
100
50
0
Relative Absorption
Premedrol
Medran
Progesteron
(int. Standard)
2
4
6
8
10
12
14
Retention time [min]

Figure 5

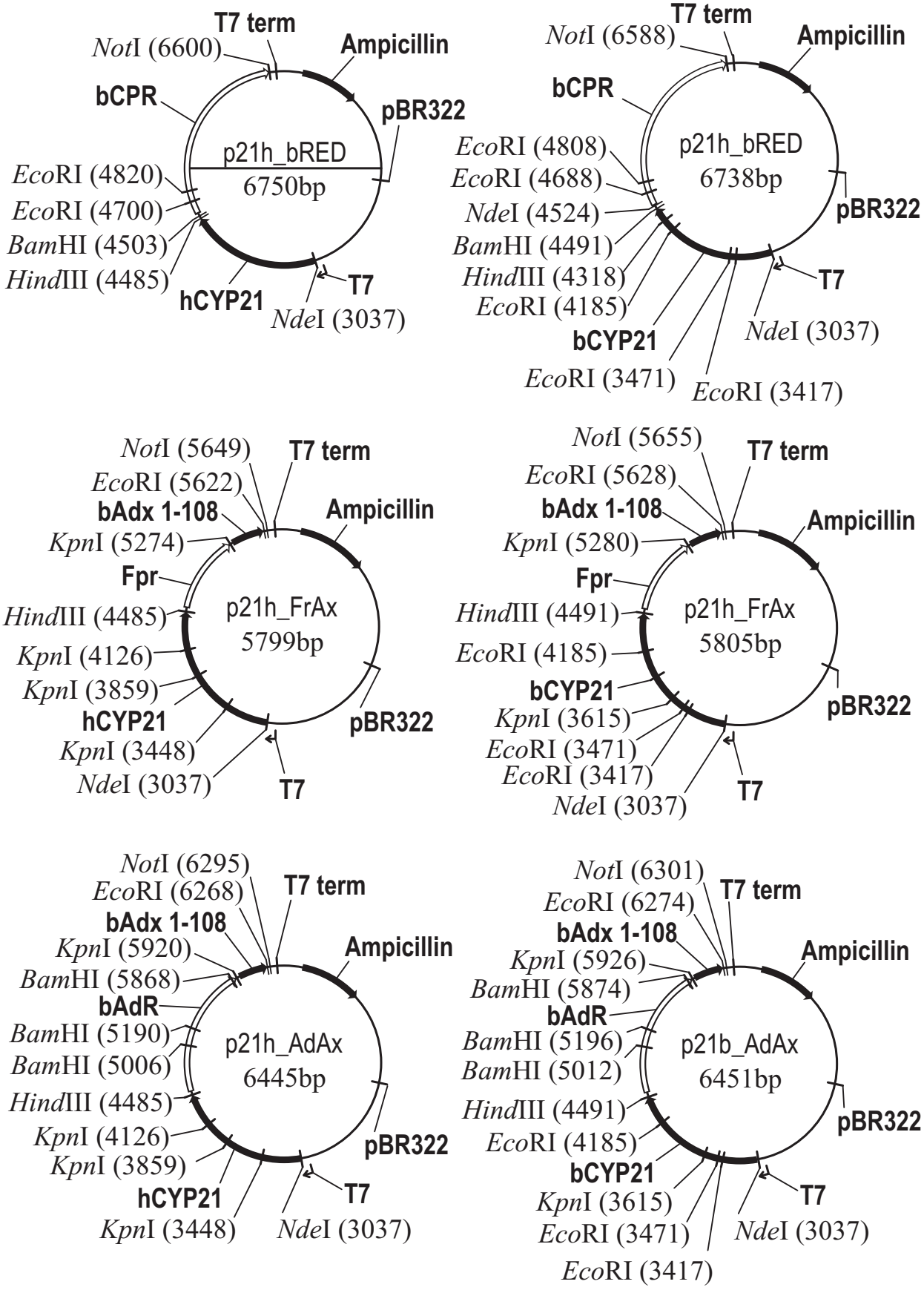

T7 term
NotI (6600)
Ampicillin
bCPR
pBR322
p21h_bRED
6750bp
EcoRI (4820)
EcoRI (4700)
BamHI (4503)
HindIII (4485)
hCYP21
T7
NdeI (3037)
T7 term
NotI (6588)
Ampicillin
bCPR
EcoRI (4808)
EcoRI (4688)
NdeI (4524)
BamHI (4491)
HindIII (4318)
EcoRI (4185)
p21h_bRED
6738bp
pBR322
T7
NdeI (3037)
bCYP21
EcoRI (3471)
EcoRI (3417)
NotI (5649)
T7 term
EcoRI (5622)
bAdx 1-108
Ampicillin
KpnI (5274)
Fpr
HindIII (4485)
p21h_FrAx
5799bp
KpnI (4126)
KpnI (3859)
hCYP21
pBR322
KpnI (3448)
NdeI (3037)
T7
NotI (5655)
T7 term
EcoRI (5628)
bAdx 1-108
Ampicillin
KpnI (5280)
Fpr
HindIII (4491)
p21h_FrAx
5805bp
EcoRI (4185)
bCYP21
pBR322
KpnI (3615)
EcoRI (3471)
EcoRI (3417)
NdeI (3037)
T7
NotI (6295)
EcoRI (6268)
T7 term
bAdx 1-108
KpnI (5920)
Ampicillin
BamHI (5868)
bAdR
BamHI (5190)
p21h_AdAx
6445bp
BamHI (5006)
HindIII (4485)
KpnI (4126)
pBR322
KpnI (3859)
hCYP21
T7
KpnI (3448)
NdeI (3037)
NotI (6301)
EcoRI (6274)
T7 term
bAdx 1-108
KpnI (5926)
Ampicillin
BamHI (5874)
bAdR
BamHI (5196)
p21b_AdAx
6451bp
BamHI (5012)
HindIII (4491)
pBR322
EcoRI (4185)
bCYP21
KpnI (3615)
T7
EcoRI (3471)
NdeI (3037)
EcoRI (3417)

Figure 5 (continued)

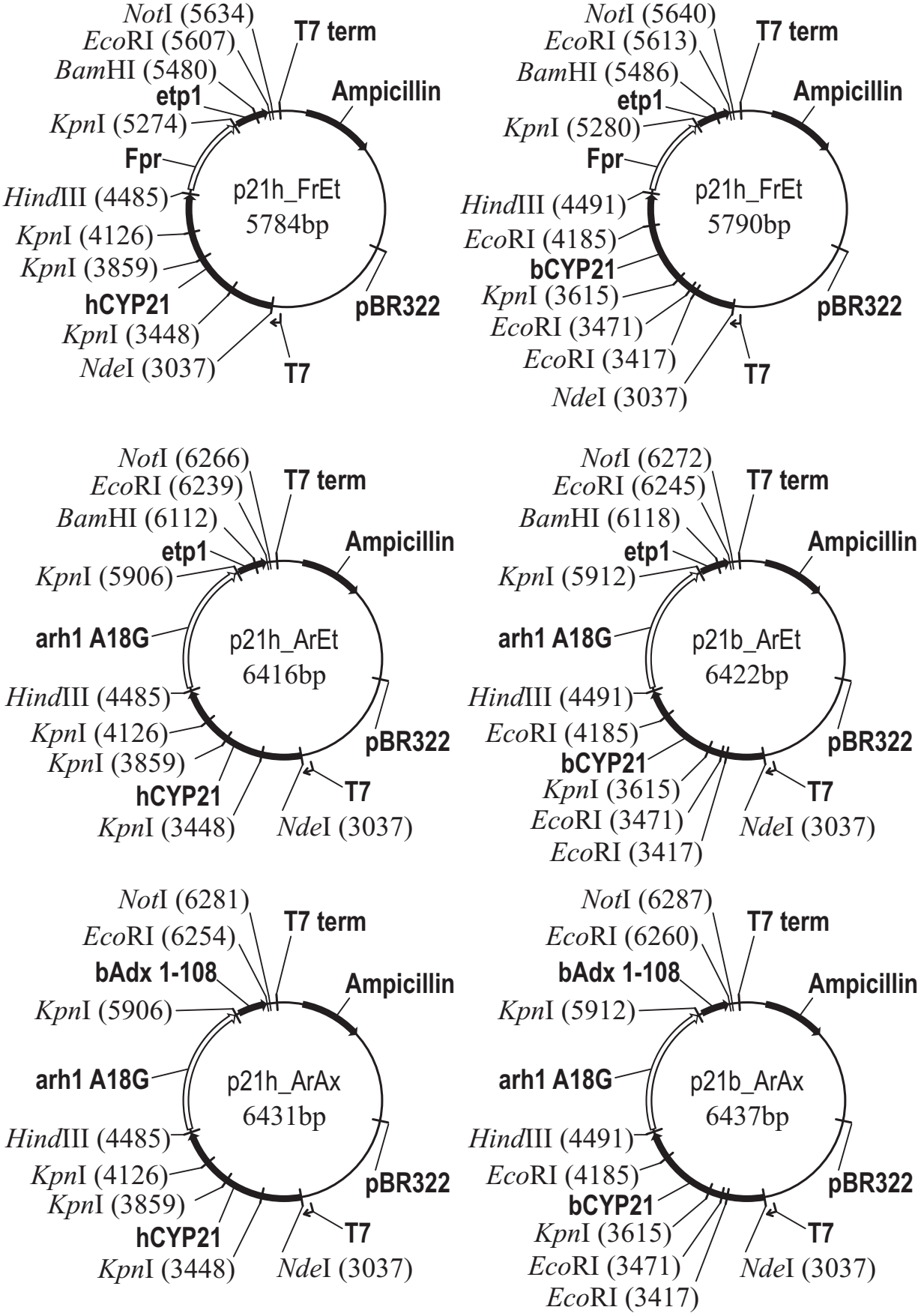


Figure 6

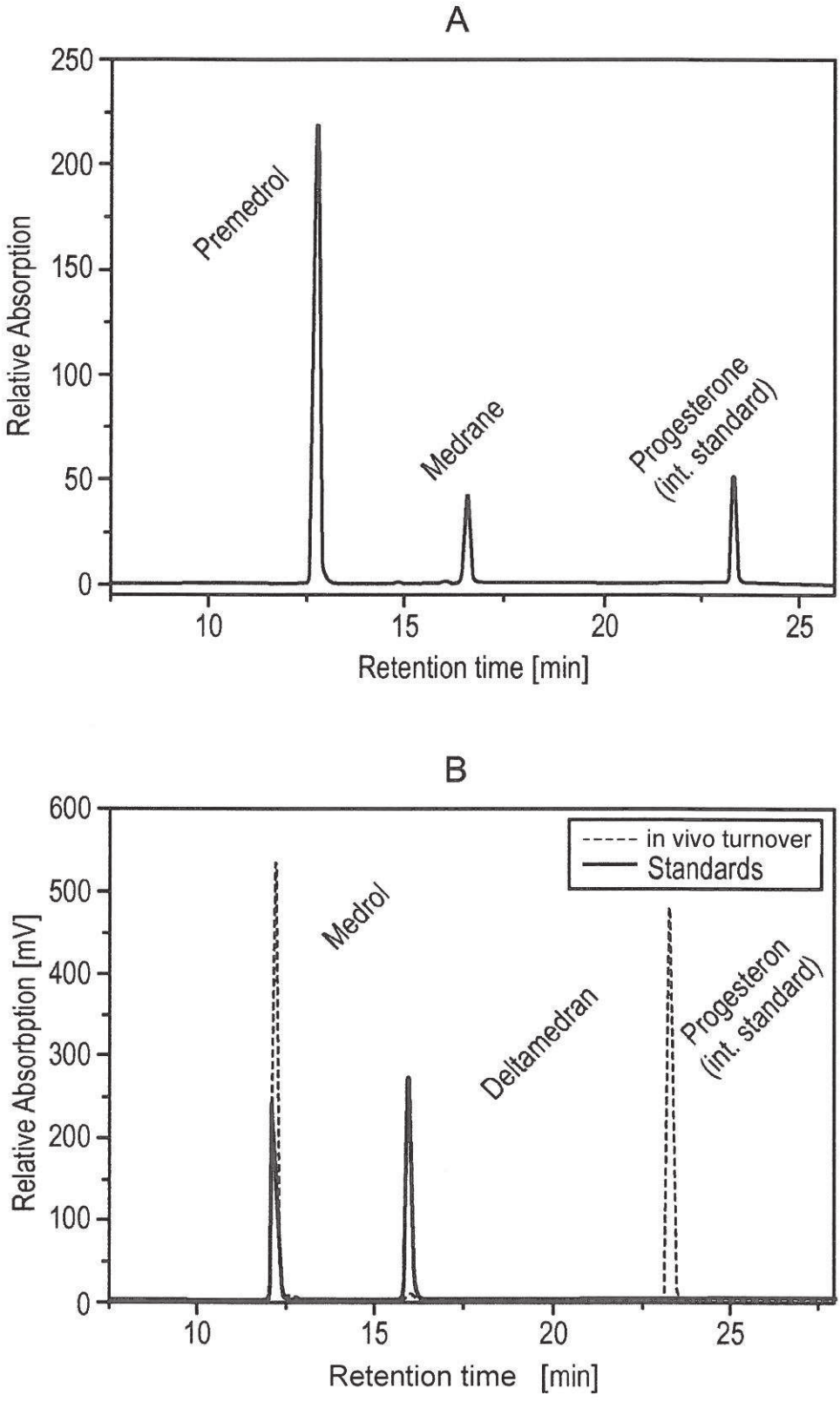
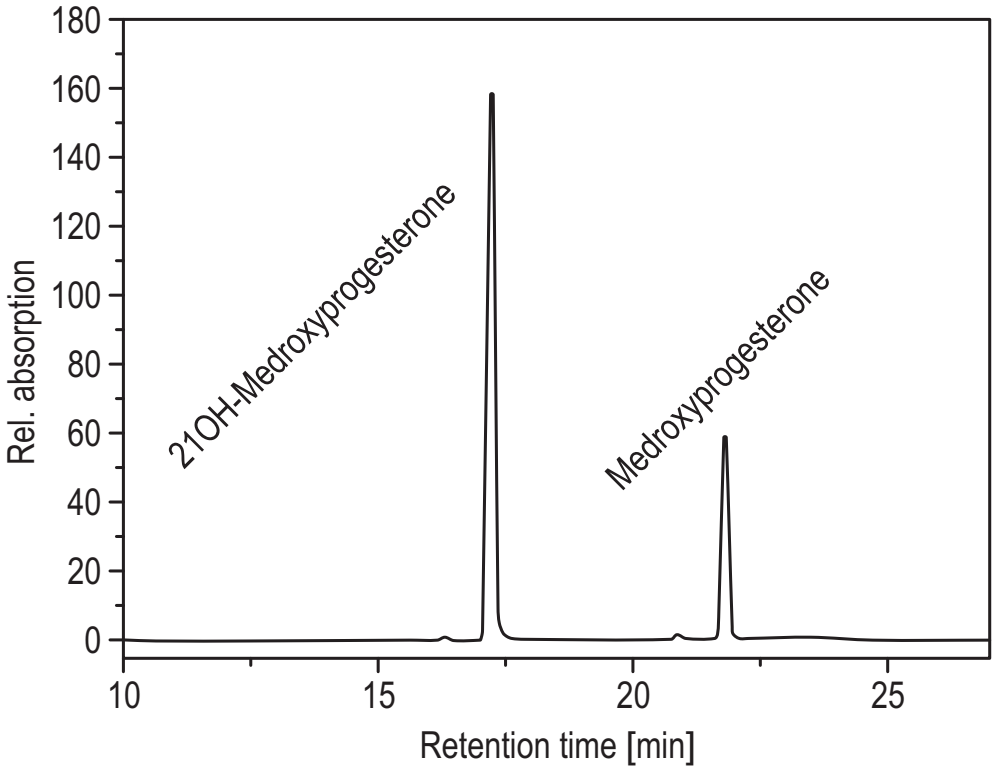

A
Relative Absorption
250
200
150
100
50
0
Premedrol
Medrane
Progesterone
(int. standard)
10
15
20
25
Retention time [min]
B
Relative Absorbption [mV]
600
500
400
300
200
100
0
----- in vivo turnover
Standards
Medrol
Deltamedran
Progesteron
(int. standard)
10
15
20
25
Retention time [min]

Figure 6 (continued)

C



D

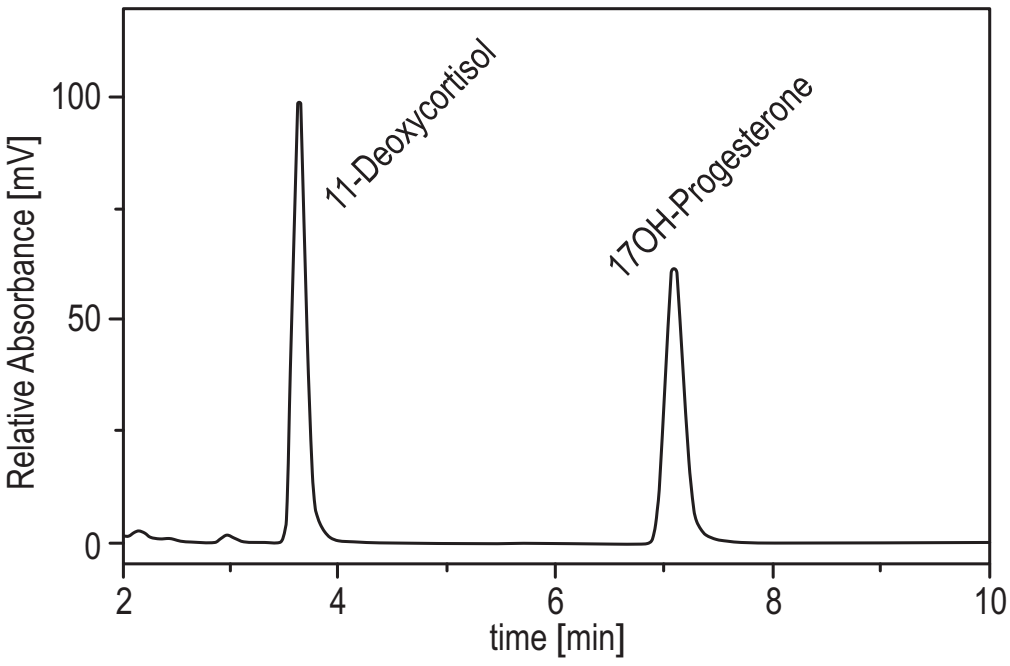


Figure 7

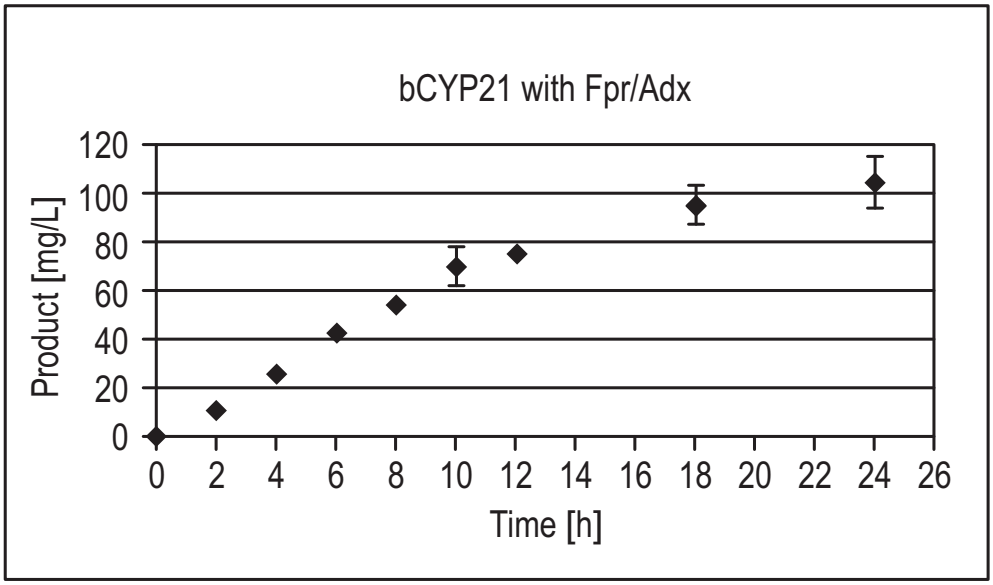

bCYP21 with Fpr/Adx
Product [mg/L]
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Time [h]

Figure 8

A

Wildtype human amino acid sequence:

*MLLLGLLLLLPLLAGARLLWNWWKLRSLHL*PPLAPGFLHLLQPDLPIYLLGLTQKFGPIYRLHL
GLQDVVVLNSKRTIEEAMVKKWADFAGRPEPLTYKLVSRNYPDLSLGDYSLLWKAHKKLTR
SALLLGIRDSMEPVVEQLTQEFCERMRAQPGTPVAIEEEFSLLTCSIICYLTFGDKIKDDNLM
PAYYKCIQEVLKTWSHWSIQIVDVIPFLRFFPNPGLRRLKQAIEKRDHIVEMQLRQHKESLVA
GQWRDMMDYMLQGVAQPSMEEGSGQLLEGHVHMAAVDLLIGGTETTANTLSWAVVFLLH
HPEIQQRLQEELDHELGPGASSSRVPYKDRARLPLLNATIAEVLRLRPVVPLALPHRTTRPS
SISGYDIPEGTVIIPNLQGAHLDETVWERPHEFWPDRFLEPGKNSRALAFGCGARVCLGEPL
ARLELFVVLTRLLQAFTLLPSGDALPSLQPLPHCSVILKMQPFQVRLQPRGMGAHSPGQSQ

Modified human amino acid sequence:

MAKKTSSKGKPPLAPGFLHLLQPDLPIYLLGLTQKFGPIYRLHLGLQDVVVLNSKRTIEEAMV
KKWADFAGRPEPLTYKLVSRNYPDLSLGDYSLLWKAHKKLTRSALLLGIRDSMEPVVEQLT
QEFCERMRAQPGTPVAIEEEFSLLTCSIICYLTFGDKIKDDNLMPAYYKCIQEVLKT WSHWSI
QIVDVIPFLRFFPNPGLRRLKQAIEKRDHIVEMQLRQHKESLVAGQWR DMMDYMLQGVAQP
SMEEGSGQLLEGHVHMAAVDLLIGGTETTANTLSWAVVFLLHHPEIQQRLQEELDHELGPG
ASSSRVPYKDRARLPLLNATIAEVLRLRPVVPLALPHRTTRPSSISGYDIPEGTVIIPNLQGAH
LDETVWERPHEFWPDRFLEPGKNSRALAFGCGARVCLGEPLARLELFVVLTRLLQAFTLLP
SGDALPSLQPLPHCSVILKMQPFQVRLQPRGMGAHSPGQSQ**HHHHHH**

Figure 8 (continued)

B

Wildtype bovine amino acid sequence:

MVLAGLLLLLTLLAGAHLLWGRWKLRNLHL PPLVPGFLHLLQPNLPIHLLSLTQKLGPVYRLR
LGLQEVVVLNSKRTIEEAMIRKWVDFAGRPQIPSYKLVSQRCQDISLGDYSLLWKAHKKLTR
SALLLGTRSSMEPWVDQLTQEFCERMRVQAGAPVTIQKEFSLLTCSIICYLTFGNKEDTLVH
AFHDCVQDLMKTWDHWSIQILDMVPFLRFFPNPGLWRLKQAIENRDHMVEKQLTRHKESM
VAGQWRDMTDYMLQGVGRQRVEEGPGQLLEGHVHMSVVDLFIGGTETTASTLSWAVAFL
LHHPEIQRRLQEELDRELGPGASCSRVTYKDRARLPLLNATIAEVLRLRPVVPLALPHRTTRP
SSIFGYDIPEGMVVIPNLQGAHLDETVWEQPHEFRPDRFLEPGANPSALAFGCGARVCLGE
SLARLELFVVLLRLLQAFTLLPPPVGALPSLQPDPYCGVNLKVQPFQVRLQPRGVEAGAWE
SASAQ

Modified bovine amino acid sequence:

MAKKTSSKGKPPLVPGFLHLLQPNLPIHLLSLTQKLGPVYRLRLGLQEVVVLNSKRTIEEAMI
RKWVDFAGRPQIPSYKLVSQRCQDISLGDYSLLWKAHKKLTRSALLLGTRSSMEPWVDQLT
QEFCERMRVQAGAPVTIQKEFSLLTCSIICYLTFGNKEDTLV HAFHDCVQDLMKTWDHWSIQ
ILDMVPFLRFFPNPGLWRLKQAIENRDHMVEKQLTRHKESMVAGQWRDMTDYMLQGVGR
QRVEEGPGQLLEGHVHMSVVDLFIGGTETTASTLSWAVAFLLHHPEIQRRLQEELDRELGP
GASCSRVTYKDRARLPLLNATIAEVLRLRPVVPLALPHRTTRPSSIFGYDIPEGMVVIPNLQG
AHLDETVWEQPHEFRPDRFLEPGANPSALAFGCGARVCLGESLARLELFVVLLRLLQAFTLL
PPPVGALPSLQPDPYCGVNLKVQPFQVRLQPRGVEAGAWESASAQ**HHHHHH**