

Supplemental Data Tables

SUPPLEMENTAL DATA TABLE 1. Mutagenic oligonucleotides

<u>Mutation/Terminal primer</u>	<u>Sense oligonucleotide</u>	<u>Antisense oligonucleotide</u>
pPGKS2	AAGATGCCGATTTGGGCGCGAATCC	--
iPGKAS1	--	GCAACACCTGGCAATTCCTTACCTTCC
17βHSD1-R38K	GCCACGTTGAAGGACCTGAAAACACAG	TTTCAGGTCCTTCAACGTGGCATAACAC
17βHSD1-R38G	GCCACGTTGGGTGACCTGAAAACACAG	TTTCAGGTCACCCAACGTGGCATAACAC
17βHSD1-R38D	GCCACGTTGGATGACCTGAAAACACAG	TTTCAGGTCATCCAACGTGGCATAACAC
17βHSD2-E116D	AGTTTTGAATGACAATGGCCCAGGAGC	TGGGCCATTGTCAATCAAAACTCCGGC
17βHSD2-E116G	AGTTTTGAATGGAAATGGCCCAGGAGC	TGGGCCATTTCCATTCAAAACTCCGGC
17βHSD2-E116R	AGTTTTGAATAGAAATGGCCCAGGAGC	TGGGCCATTTCTATTCAAAACTCCGG
17βHSD2-E116G+N117R	TTGAATGGAAGAGGCCAGGAGCTGAG	TCCTGGGCCTCTTCCATTCAAAACTCC
11βHSD2-17βHSD2 chimera	TGCCTTCTGATCCACCGGCAGGCGCTGC	CAGCGCCTGCCGGTGGATCAGAAGGCAG

SUPPLEMENTAL DATA TABLE 2. Conditions for equilibrium kinetics experiments

<u>Medium</u>	<u>[³H]-Estrone</u>		<u>[¹⁴C]-Estradiol</u>		<u>E1:E2 Ratio</u>
	<u>cpm</u>	<u>μM</u>	<u>cpm</u>	<u>μM</u>	
Complete	1,000,000	0.23	640,000	0.93	20:80
2-Deoxyglucose	600,000	0.45	480,000	0.70	40:60

SUPPLEMENTAL DATA TABLE 3. Representative purification of wild-type 17βHSD1 from 1 liter culture of *E coli*

Step Of Purification	Total Volume (ml)	Protein Conc. (mg/ml)	Total Protein (mg)	Activity (U/ml)	Total Activity (U)	Specific Activity (U/mg)	Fold Purification	Yield (%)
Crude Extract	150	12.87	1931.1	102.3	15345	7.9	1	100
Heat treatment	310	0.64	199.6	34.1	10570	52.9	6.7	68.9
Red Agarose Gel	10	1.23	12.3	120.9	1209	98.3	1.85	11.4

SUPPLEMENTAL DATA TABLE 4. Residues in cofactor binding regions and structural elements for 17βHSD1, 11βHSD1, and gluconate 5-dehydrogenase from x-ray structures and of 17βHSD2 and 11βHSD2 by alignment.

		LOOP1	BETA1	LOOP2	ALPHA1	LOOP3	BETA2	LOOP4	ALPHA2	LOOP5	BETA3	LOOP6	ALPHA3	Cofactor
HSD17B1	RX	ART	VVLIT	GcSS	GIGLHLAVRLAS	DPSQSF	KVYATL	RD	LKTQGRWLWEAARAL	ACPPGS	LETLQ	LDVRD	SKSVAAARERT	NADP
HSD17B2	OX	DQK	AVLVT	GgDC	GLGHALCKYLDE	LGF	TVFAGV	LNENG	PGAEELRRT	CSPRL	SVLQ	MDITK	PVQIKDAYSKDAAM	NAD
HSD11B1	RX	QGK	KAVIVT	GASK	GIGREMAYHLAK	MGA	HVVVTA	RS	KETLQKVVSHCLE	LGAAS	AHYIA	GTMED	MTFAEQFVAQAGKL	NADP
HSD11B2	OX	ATR	AVLIT	GcDS	GFGKETAKKLD	MGS	TVLATV	LELNS	PGAIELRSC	CSPR	LRLQ	MDLTK	PGDISRVLEFTKAHT	NAD
Ga5DH2	OX	KGK	IALVT	GASY	GIGFAIASAYAK	AGA	TIVFND	IN	QELVDRGMAAYKA	AGIN	AHGTV	CDVTD	EDGIQAMVAQIESE	NADP