

Supplementary Materials

for

Discovery of hCES2A inhibitors from *Glycyrrhizae Inflatae* via combination of docking-based virtual screening and fluorescence-based inhibition assays

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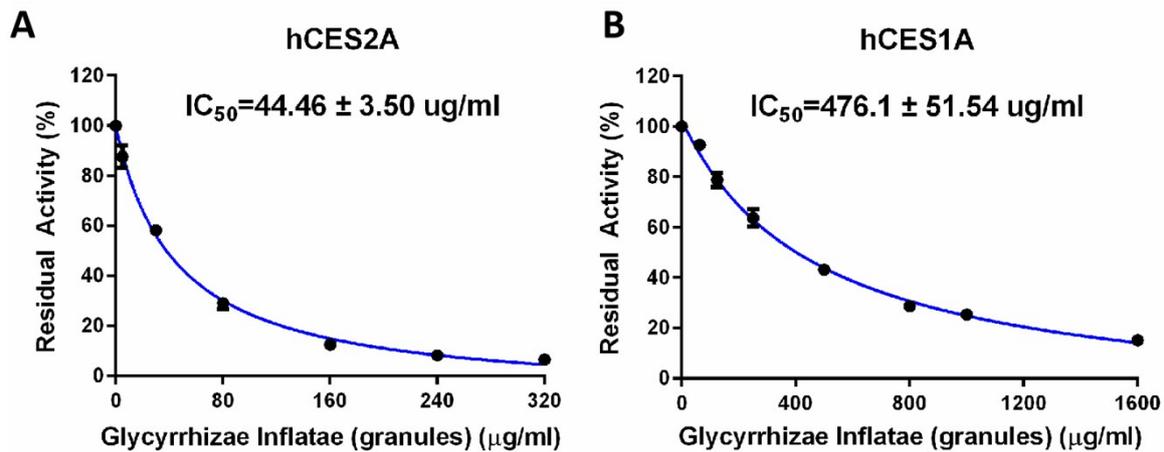


Fig. S1 The dose-inhibition curves for Glycyrrhizae Inflatae (granules) against hCES2A-mediated FD hydrolysis in HLM (A) and hCES1A-mediated DME hydrolysis in HLM (B). All data were shown as mean \pm SD of triplicate assays.

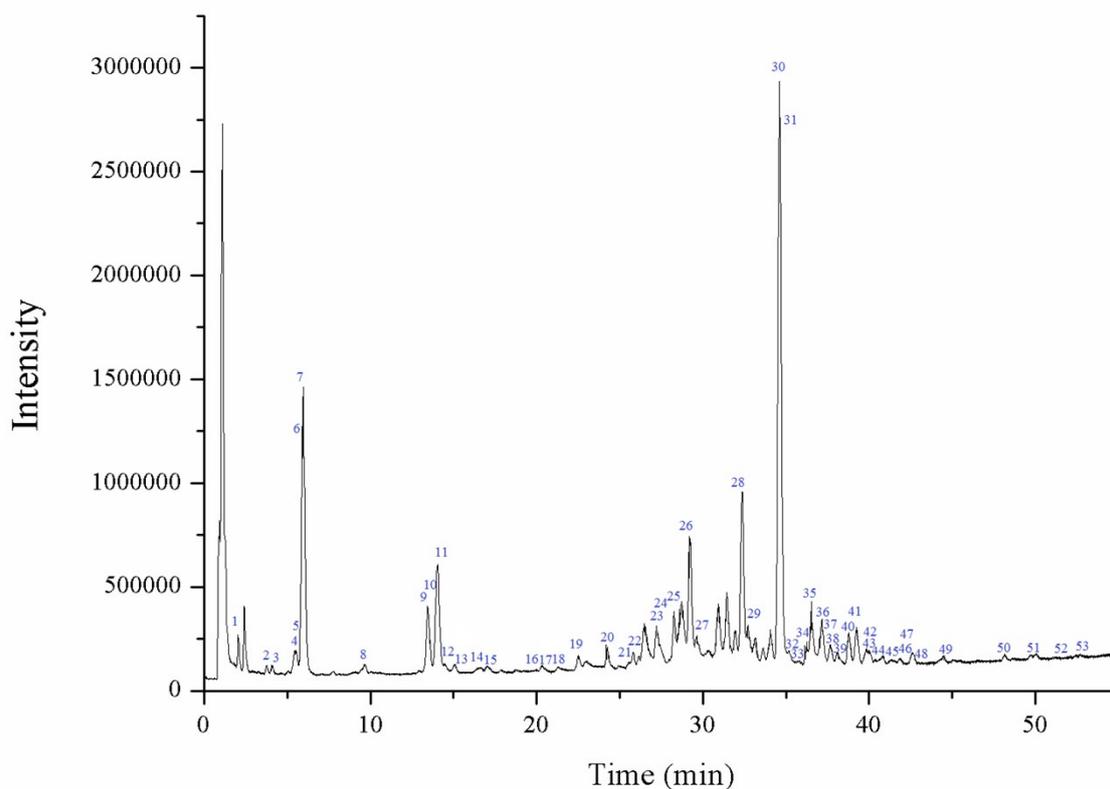


Fig. S2. Total ion chromatogram of *Glycyrrhizae Inflatae* granule in negative ion mode.

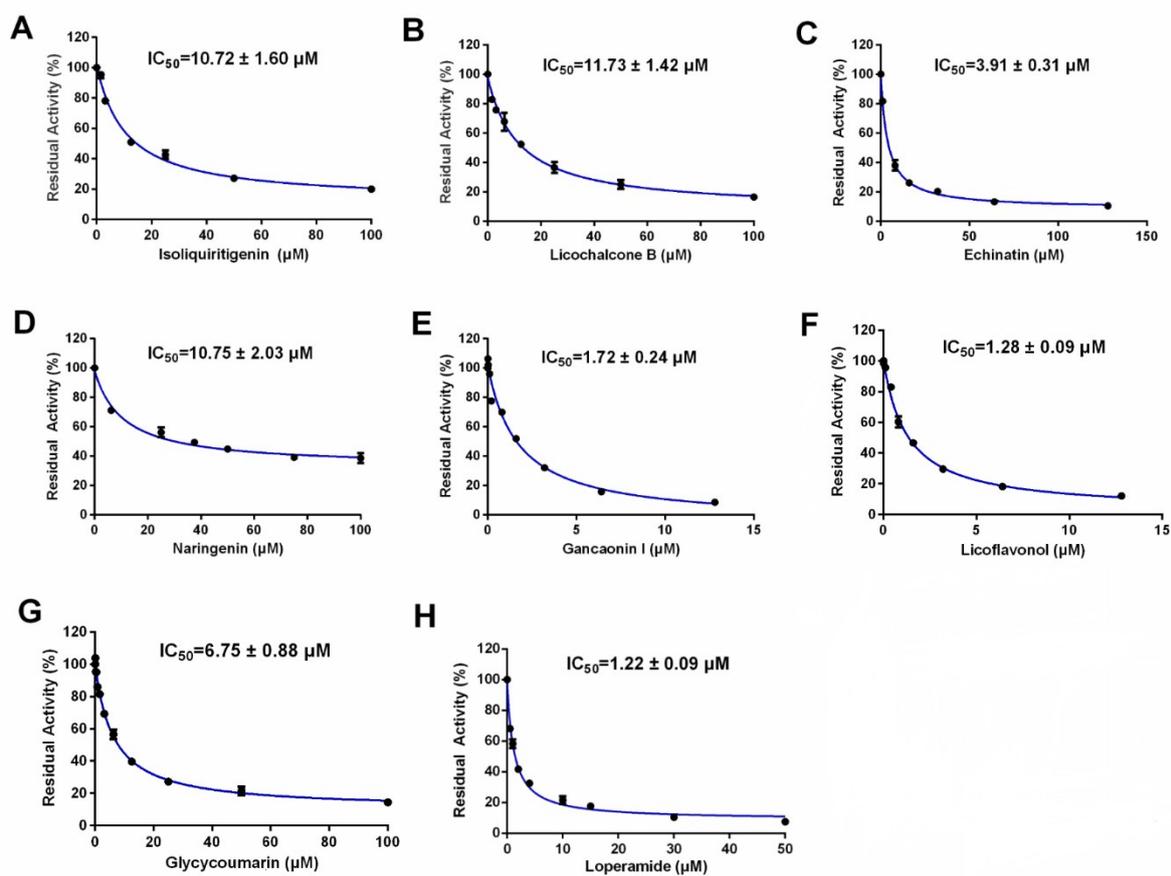


Fig. S3 The dose-inhibition curves of Isoliquiritigenin (A), Licochalcone B (B), Echinatin (C), Naringenin (D), Gancaonin I (E), Licoflavonol (F), Glycycoumarin (G) and Loperamide (H) against hCES2A-mediated FD hydrolysis. All data were shown as mean \pm SD of triplicate assays.

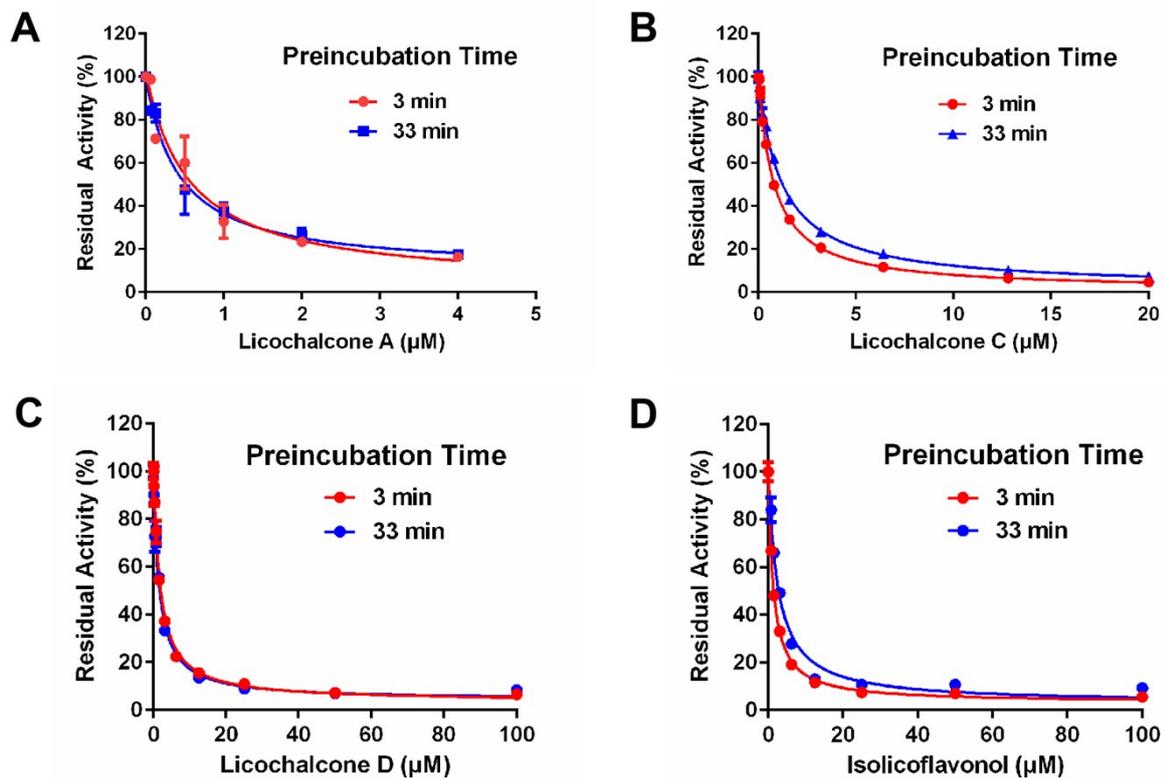


Fig. S4 Time-dependent inhibition assays of Licochalcone A (A), Licochalcone C (B), Licochalcone D (C) and Isicoflavonol (D) on hCES2A using FD as the substrate. All data were shown as mean \pm SD of triplicate determinations.

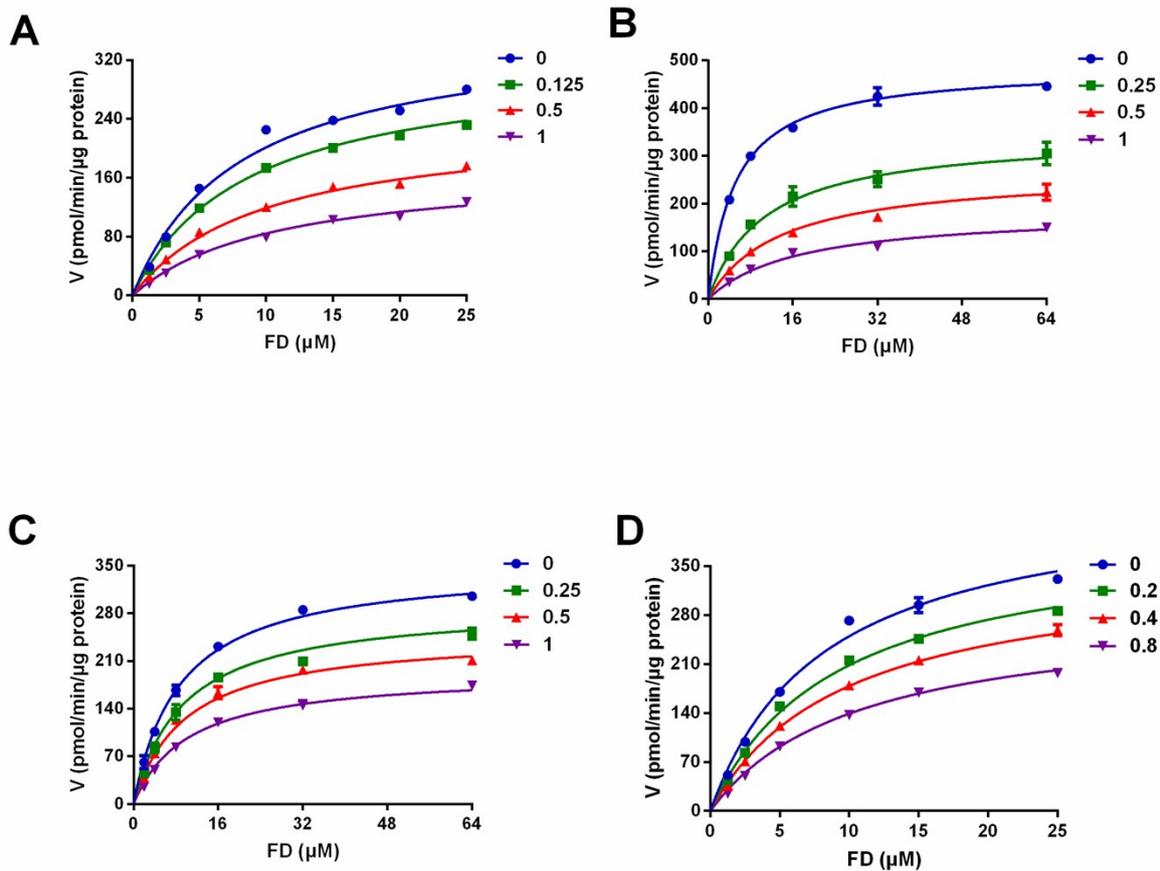


Fig. S5 The inhibition kinetic plots of Licochalcone A (A), Licochalcone C (B), Licochalcone D (C) and Isolicoflavonol (D) on hCES2A using FD as the substrate. All data were shown as mean \pm SD of triplicate determinations.

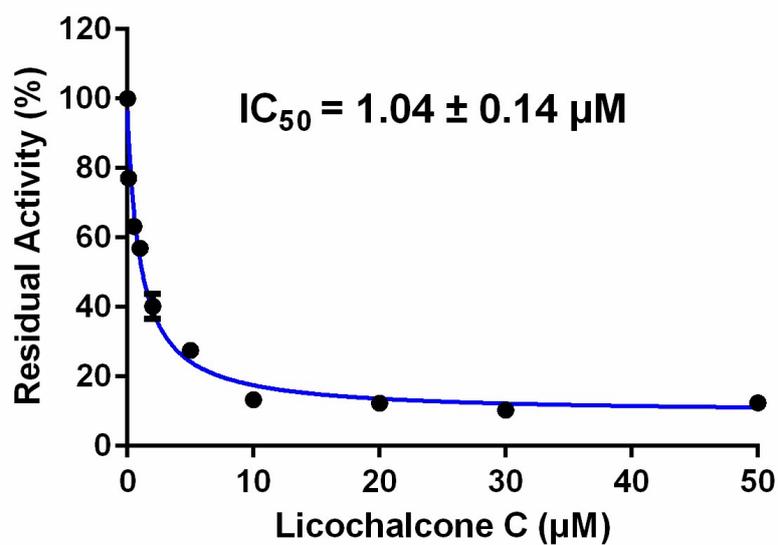


Fig. S6 Dose-inhibition curve of Licochalcone C against intracellular hCES2A in living HepG2 cells. All data were shown as mean ± SD of triplicate determinations.

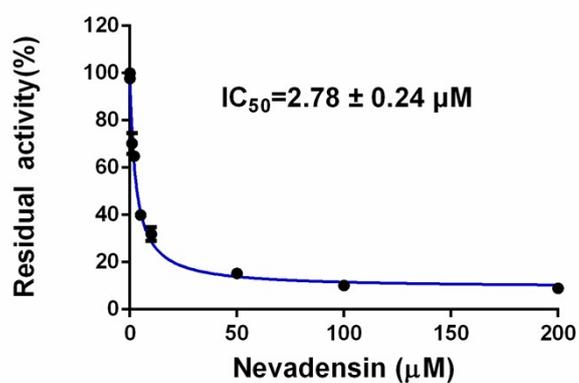


Fig. S7 Dose-inhibition curves of nevadensin against hCES1A-mediated DME hydrolysis in HLM. All data were shown as mean ± SD of triplicate assays.

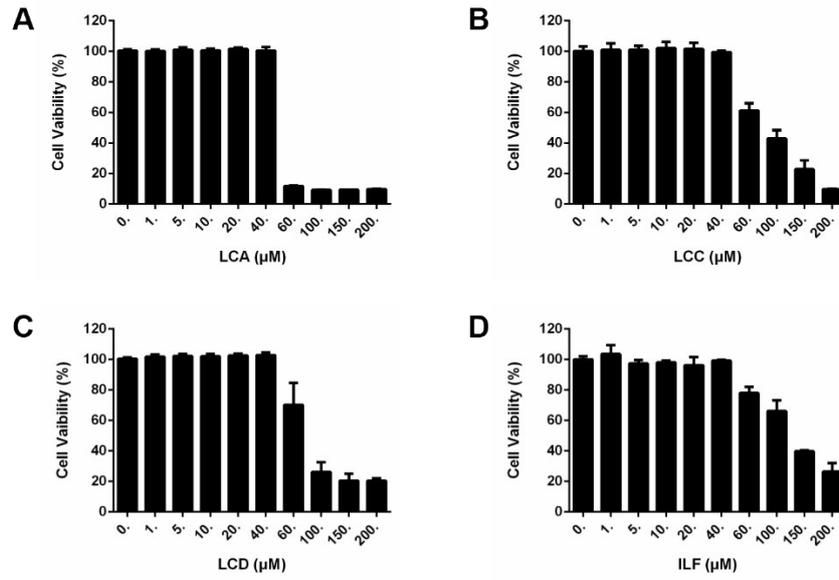


Fig. S8 Cytotoxicity assay of Licochalcone A (A), Licochalcone C (B), Licochalcone D (C) and Isicoflavonol (D) in HepG2 cells. All data were shown as mean \pm SD of triplicate determinations.

Table S1. The predicted binding energy and CDOCKER interaction energy values of 53 constituents from licorice and Loperamide.

No.	Compound	Binding Energy (kcal/mol)	CDOCKER Interaction Energy (kcal/mol)	IC ₅₀ (μM)
1	Glucoliquiritin apioside	> 0	--	--
2	Schaftoside	> 0	--	--
3	Glucoliquiritin	> 0	--	--
4	Neoliquiritin*	> 0	--	>100
5	Glucosoliquiritin	> 0	--	--
6	Liquiritin apioside*	> 0	--	>100
7	Liquiritin*	-23.4146	-40.7349	>100
8	5-hydroxyliquiritin	-40.0189	-47.0485	--
9	Isoliquiritin apioside	-43.3705	-50.8770	--
10	Isoliquiritin	-173.228	-55.6469	--
11	Liquiritigenin*	> 0	--	>100
12	Licochalcone B*	-166.808	-56.216	11.73 ± 1.42
13	Neoisoliquiritin	-72.1795	-38.319	--
14	Licorice glycoside B	-88.6195	-24.1624	--
15	Licorice glycoside A	> 0	--	--
16	LicoricesaponinO4	ND	--	--
17	naringenin*	-229.693	-49.5519	10.75 ± 2.03
18	Genistein	> 0	--	--
19	Echinatin*	-265.935	-49.8474	3.91 ± 0.31
20	Licorice saponin J2	> 0	--	--
21	24-Hydroxy-licorice-saponin A3	ND	--	--
22	22-Hydroxy-licorice-saponin G2	> 0	--	--
23	Isoliquiritigenin*	-201.558	-49.9137	10.72 ± 1.60
24	Licoricesaponin M3	ND	--	--
25	Formononetin*	-65.4651	-27.327	>100
26	22β-Acetoxyglycyrrhizic acid	ND	--	--
27	23-Hydroxyl licorice saponin E 2	ND	--	--
28	licoricesaponin G 2	ND	--	--
29	22β-Acetoxylicorice saponin J 2	ND	--	--
30	Glycyrrhizic Acid*	ND	--	>100
31	licoricesaponin A3	ND	--	--
32	Licochalcone D*	-98.7995	-45.2915	0.94 ± 0.11
33	Gancaonin L	-78.5992	-36.9273	--

34	Licoricesaponin b2	ND	--	--
35	LicoricesaponinH2	ND	--	--
36	Gancaonin M	-74.1977	-34.1285	--
37	Glycoumarin*	-174.782	-55.4037	6.75 ± 0.88
38	Licoagrodione	-137.442	-36.0848	--
39	Licoricesaponin K2	ND	--	--
40	Apioglycyrrhizin	ND	--	--
41	Gancaonin C	-188.469	-46.637	--
42	Licochalcone C*	-257.661	-55.0714	0.39 ± 0.04
43	Licoricesaponin C2	ND	--	--
44	Licochalcone A*	-281.533	-55.1037	0.54 ± 0.06
45	Isolicoflavonol*	-294.014	-58.7403	0.60 ± 0.08
46	Licoflavonol*	-292.033	-53.3065	1.28 ± 0.09
47	Isoglycyrol	> 0	--	--
48	Glycyrrhetic acid 3-O-glucuronide	ND	--	--
49	Gancaonin G	-172.989	-34.4663	--
50	kanzonol R	> 0	--	--
51	Licofuranone	-35.9798	-34.3298	--
52	Gancaonin I*	-215.515	-54.1744	1.72 ± 0.24
53	Glycyrrhetic acid	> 0	--	--
--	Loperamide	-69.9112	-34.2231	1.22 ± 0.09

Note: ND represented that the small molecule could not be docked into the active cavity of hCES2A. Twenty-four ligands with binding energy less than 0 were further screened by CDOCKER analyses. Seventeen compounds with lower binding energy values and CDOCKER interaction energy values than LPA were shown in blue.

Table S2. AutoDock vina results of Licochalcone C docked with hCES2A

Binding Site	Binding affinity (kcal/mol)
site I	-8.2
site II	-8.0

S1. The ethics policy statement and product report provided by BioreclamationIVT.



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BioreclamationIVT Ethics Policy Statement

BioreclamationIVT has established itself as the leading industry supplier of quality ADME-Tox products to pharmaceutical, government, and academic institutions. BioreclamationIVT maintains strict adherence to all applicable ethical guidelines and regulations, as well as an inherent respect for the origin and dispositions for the materials we possess. BioreclamationIVT does not traffic in human tissue, and as such will only reimburse verified non-profit providers of research tissue for appropriate costs of tissue procurement, preparation, and transportation. BioreclamationIVT has the utmost respect and appreciation for all donated tissues and thus strives to apply the best available technologies, quality control, and business practices to ensure the best and most efficient use of the tissues received from all sources. Unprocessed tissue is never offered for resale. BioreclamationIVT will only market tissue derivatives such as cells and sub-cellular fractions. BioreclamationIVT does not accept any tissues or organs for cell processing that are qualified for transplant. BioreclamationIVT only procures tissue from within the United States, and categorically refuses offers of tissue/organs from any country other than the United States, since effective procurement and informed consent procedures are often lacking or not consistent with the practices and standards of our company. In keeping with this practice, BioreclamationIVT has appropriate procedures in place not only to safeguard the confidentiality of tissue sources, but to ensure the anonymity of any information shared with researchers related to such tissues. BioreclamationIVT educates all company personnel to treat the tissue and organs that we receive with the respect and dignity accorded to its' human origin.

Dan Dryden
Director, Product Operations
BioreclamationIVT

**InVitroCYP™ M-class 50-donor Mixed Gender Pooled
Human Liver Microsomes, 10 mg**

Product Number: X008067

Lot Number IHG

Storage Conditions -70°C

Test Results

Specification	Result
20-26 mg/mL protein concentration	20.9 mg/mL
≥0.220 nmol/mg total P450 concentration	0.347 nmol/mg

Lot Characterization Results

Assay	Enzyme activity
ECOD: total rate of formation of 7-HC and metabolites	292 pmol/min/mg
UGT: rate of formation of 7-hydroxycoumarin glucuronide	1758 pmol/min/mg
CYP1A2: rate of formation of acetaminophen	292 pmol/min/mg
CYP2A6: total rate of formation of 7-HC and metabolites	191 pmol/min/mg
CYP2B6: rate of formation of hydroxybupropion	105 pmol/min/mg
CYP2C8: rate of formation of desethylamodiaquine	950 pmol/min/mg
CYP2C9: rate of formation of 4'-methylhydroxytolbutamide	111 pmol/min/mg
CYP2C19: rate of formation of 4'-hydroxymephenytoin	37.9 pmol/min/mg
CYP2D6: rate of formation of dextroprphan	55.3 pmol/min/mg
CYP2E1: rate of formation of 6-hydroxychlorzoxazone	474 pmol/min/mg
CYP3A4: rate of formation of 6β-hydroxytestosterone	899 pmol/min/mg
rate of formation of 1-hydroxymidazolam	335 pmol/min/mg

Kinetic Parameters:

CYP	1A2	2A6	2B6	2C8	2C9	2C19	2D6	2E1	3A4 Tes*	3A4 Mid	UGT 1A1	UGT 1A4	UGT 1A6	UGT 1A9	UGT 2B7
K_m	117	1.65	89.6	1.14	208	39.2	12.0	188	105	8.35	14.8	31.7	1870	53.3	2749
V_{max}	0.9	0.954	0.56	1.53	0.043	0.025	0.24	1.01	5.10	2.28	3.64	4.01	44	8.59	9.06
Clint	7.72	578	6.3	1349	0.2	0.6	19.8	5.39	48.7	274	246	126	23.5	161	3.29

K_m is recorded as μM . V_{max} is recorded as nmol/min/mg protein. Clint is recorded as $\mu\text{L}/\text{mg}/\text{min}$.
* Testosterone at 0.1 mg/mL

Updated to include K_m , V_{max} and Cl_{int} results

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Donor Demographics, as reported to BioreclamationIVT

Gender	Age	Race	Cause of death	Height	Weight	Social history	Medical history	Serology testing					
								EBV	RPR	CMV	Hepatitis B	Hepatitis C	HIV
F	41	H	Stroke	66"	79 Kg	No ETOH, Tobacco or drug use	Appendectomy 16 yrs ago. HIE at birth, 2nd to placental abruption, w/ IDCP, Trach for bronchomalacia at 5 mos; Nissan/g-tube at 2 mos, T&A, PE tubes, seizure disorder, last seizure 2 wks ago, allergy to propofol w/resp distress, Meds: coenzyme Q10, Keppra	Not reported	Neg	Pos	Neg	Neg	Neg
F	5	C	Anoxia	3'8"	40 lb	No ETOH, Tobacco or drug use		IgG +	Neg	Pos	Neg	Neg	Neg
F	38	C	Head Trauma; 2nd to Blunt Injury	5'4"	164lb	ETOH: 4-5 drinks/day x 23yrs; Tobacco: 1 ppd since teen; no drug use.	Circulation problems suggesting signs of MS Rib fractures, contusions, pneumothorax, chest tube, occipital dislocation, quadriparesis, positive sputum culture for strep pneumo.	Not reported	Neg	Pos	Neg	Neg	Neg
F	35	C	Blunt Injury; 2nd to MVA	68"	196 lb	No ETOH; Tobacco: 3-4 cig/day; Marijuana daily	Appendectomy, hysterectomy, partial gastrectomy, bleeding ulcers, HTN, renal insuff - no meds; Med: Lasix	Not reported	Neg	Neg	Neg	Neg	Neg
F	77	C	CVA	63"	58 Kg	No ETOH, Tobacco or drug use	Heterozygous leiden factor 5 deficiency, HTN x 5yrs, multiple fractures and sxs - cervical, clipped nerve, shoulder, thumb, lumbar infusion, knee replacement, pituitary adenoma 1 yr ago, Pos TB skin test - not sure when cleared. Some type of hepatitis after meno., cellulitis. Meds: vitamins, neurontin, lynca, vicodin, docazosin, deflucan, coumadin, DVT's, fluoxetine, zocov, zeltia.	Not reported	Neg	Pos	Neg	Neg	Neg
F	58	C	CVA	65"	88 Kg	ETOH: 1-2 drinks/yr; tobacco: 1-2ppd x 30yrs - quit 10 yrs ago; no drug use		Not reported	Neg	Pos	Neg	Neg	Neg

Caution: This product was prepared from fresh human tissue. Treat all products containing human-derived materials as potentially infectious, as no known test methods can offer assurance that products derived from human tissues will not transmit infectious agents.

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Gender	Age	Race	Cause of death	Height	Weight	Social history	Medical history	EBV	RPR	CMV	Hepatitis B	Hepatitis C	HIV
F	52	C	Cardiac Arrest; 2nd to Head trauma/Fall	64"	79 Kg	No ETOH, Tobacco or drug use	High cholesterol, diabetes NIDDM x 10yrs - oral meds	Not reported	Neg	Pos	Neg	Neg	Neg
F	58	C	Anoxia; 2nd to pulm embolism	64"	78 Kg	ETOH: 6yrs extensively - 1 gallon of vodka QD, quit 1-1/2 yrs ago; Tobacco: 40 pack yrs; Drugs: Marijuana 1 x 1 month	Positive Tox screen (ETOH/ anti-depressants), bipolar. Meds: antidepressant	Not reported	Neg	Not reported	Neg	Neg	Neg
F	40	B	ICH; 2nd to SAH & cerebral edema	70"	115Kg	ETOH: social, Tobacco: 1ppd x 20yrs, Drugs: possible marijuana	Diabetes - inconsistent w/meds, HTN x 10yrs - non-compliant, bipolar, pituitary tumor 8 yrs ago, renal cell carcinoma - kidney; Meds: Coreg, effexor, ferous sulfate, novolog-insulin, lipitor, lisinopril, norvasc, clonidine	Not reported	Neg	Pos	Neg	Neg	Neg
F	73	C	Stroke	66"	74 Kg	No ETOH or drug use; Tobacco - quit 37 yrs ago	HTN, GERD	Not reported	Neg	Pos	Neg	Neg	Neg
F	65	C	CVA/Stroke; 2nd to ICH/Stroke	63"	87 Kg	No ETOH, Tobacco or drug use	HTN-med compliant, knee replacement 1 yrs ago, back sx for ruptured disk 8 - 10 yrs ago	Not reported	Neg	Pos	Neg	Neg	Neg
F	35	C	SIGSWH	64"	142lb	ETOH: Binge drinker - quit 1 yr ago; Tobacco: 2 cigs/day; Drugs: IV drug abuse 1 yr ago, snorted cocaine 9yrs ago, marijuana 3x/wk for years.	No other medical history	Not reported	Neg	Pos	Neg	Neg	Neg
F	78	C	CVA	58"	57 Kg	Tobacco: 1ppd x 40 yrs - quit; No ETOH or drug use	HTN x 1 yr, high cholesterol, bursitis L hip	Not reported	Neg	Pos	Neg	Neg	Neg
F	56	C	ICH-Stroke	5'5"	152lb	ETOH: Couple glasses wine a day x 3yrs; tobacco: 1ppd x 40 yrs, no drug use	Cardiac disease, Rheumatoid fever 47 yrs ago (treated w/rest)	IgG+	Neg	Pos	HbsAb+	Neg	Neg
F	56	C	ICH	69"	245lb	No ETOH, Tobacco or drug use	Diabetes and HTN >10yrs - compliant, mild stroke 3 mos ago. Meds: Insulin, HTN	Not reported	Neg	Pos	Neg	Neg	Neg

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Gender	Age	Race	Cause of death	Height	Weight	Social history	Medical history	EBV	RPR	CMV	Hepatitis B	Hepatitis C	HIV
F	48	C	CVA	66"	87 Kg	No ETOH, Tobacco or drug use	Peripheral artery disease, ESRD, HTN, hyperlipidemia, DM, CAD, AV fistula occlusion, hemodialysis. Meds: Amoxicillin, clopidogrel, carvedilol, folic acid, glimepiride, sertraline, zolpidem, clonidine, amlodipine	Pos	Neg	Pos	Neg	Neg	Neg
F	39	C	Anoxia; 2nd to Cardiac arrest	63"	90 Kg	ETOH: occasionally; Tobacco: 1/2 ppd x 10yrs; Drugs: abused prescription narcotics, smoked marijuana	HTN x 3yrs: non-compliant	Pos	Neg	Pos	Neg	Neg	Neg
F	49	A	CVA/Stroke	55"	99 lb	No ETOH, Tobacco or drug use	HTN, DM (6-10 yrs), c-section 8 yrs ago; Meds - possible Anti-HTN.	Not reported	Neg	Not reported	Neg	Neg	Neg
F	62	C	CVA	64"	179lb	No ETOH or drug use; Tobacco: >20 pack yrs - quit 6 months ago.	T2D (NIDDM) x15yrs - unknown if compliant, HTN 0-5yrs - compliant, CHF, thyroid disease, ESRD with dialysis, COPD, emphysema, colonoscopy with cauterization (no cancer), Steven Johnson syndrome - 3.5yrs ago, dialysis 3x/wk x 4yrs, born w/1 kidney - atrophic	IgG+	Neg	Pos	Neg	Neg	Neg
F	43	C	Anoxia	61"	80 Kg	No ETOH; Tobacco: 1/2 ppd x 25 yrs; Drugs: Prescription pill abuse, tox screen ps for benzo & opiate, bruised track marks noted, denied IVDA.	HTN, chronic back pain, rectal ulcer, IBS, ovarian cancer 16 yrs ago - treated w/ chemo and radiation, UTI, MI x2, seizure secondary to detox, tonsillectomy, hysterectomy, oophorectomy, cardiac cath 1 yr ago; Meds: methadone, clonidine, neurontin.	Not reported	Neg	Not reported	Neg	Neg	Neg
F	74	C	Head Trauma	65"	53 Kg	ETOH: 3-4 glasses of wine/day x 30yrs; No tobacco or drug use	No history provided.	Pos	Neg	Pos	Neg	Neg	Neg

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F	71	C	ICH	67"	132Kg	ETOH: 3 glasses wine QDx50 yrs; Tobacco - quit 25 yrs ago; no drug use	Knee replacement, hysterectomy, 5 yrs ago-long plane fit-DVT in leg - progressed to PE. Meds: Coumadin, Paxil	Not reported	Neg	Pos	Neg	Neg	Neg
F	63	C	Anoxia; 2nd to Cardio-vascular	69"	100 Kg	No ETOH, Tobacco or drug use	HTN - med compliant, unknown med names	Not reported	Neg	Pos	Neg	Neg	Neg
F	73	C	CVA	59"	60 Kg	ETOH- Occasional; Tobacco: 1ppd x 20-30 yrs; no drug use	High Cholesterol, hysterectomy 45 yrs ago, benign cyst in breast. Meds: Lipitor.	Not reported	Neg	Neg	Neg	Neg	Neg
F	70	C	ICH/Stroke	66"	89 Kg	No ETOH; Tobacco: 1 ppd x 25-30yrs - quit 3 months ago; no drug use	Hypothyroidism, HTN, Hyperlipidemia, knee surgery 8 mos. ago, extremely dry skin. Meds: cortisone, synthroid, aspirin, lipitor	Not reported	Neg	Pos	Neg	Neg	Neg
M	49	C	CVA	75"	110Kg	Tobacco: 2ppd x 30 yrs; No ETOH; smoked marijuana and cocaine abuse	Untreated HTN, chronic back pain, appendectomy; Meds: Ambien	Not reported	Neg	Pos	Neg	Neg	Neg
M	49	C	CVA	70"	256lb	No ETOH, Tobacco or drug use	HTN x 10yrs, Diabetes 6-10yrs, CAD. Meds: anti-HTN, diabetes	Not reported	Neg	Neg	Neg	Neg	Neg
M	54	C	ICH	73"	125Kg	ETOH: quit 3 yrs ago; Tobacco: 1 -2 ppd x 35 yrs; No drug use.	2x GSW injuries, gall bladder & appendix removed	Not reported	Neg	Neg	Neg	Neg	Neg
M	29	C	GSW	69"	94 Kg	ETOH: Alcoholic; Tobacco: 1 ppd x 8 yrs; no drug use	Depression	Not reported	Neg	Neg	Neg	Neg	Neg
M	85	C	Head Trauma; 2nd to Fall from roof	68"	251lb	No ETOH or Tobacco use. No drug use reported, but tox screen positive for benzos and opiates	NIDDM >10yrs, HTN >10yrs - compliant, ruptured bladder - 35 yrs ago, ruptured gallbladder 3 yrs ago, Cataracts-bilat. Meds: Oral meds for diabetes, HTN	IgG+	Neg	Pos	Neg	Neg	Neg
M	56	B	CVA	71"	103Kg	No ETOH, Tobacco or drug use	Tooth extraction, CABG, end stage renal disease x 7 yrs, CAD, pneumonia 1 yr ago, HTN x 25 yrs, enlarged heart >15 yrs, IDDM, heart bypass 2 yrs ago, high blood pressure > 25 yrs, previous pos skin test for TB	Not reported	Neg	Pos	Neg	Neg	Neg

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Gender	Age	Race	Cause of death	Height	Weight	Social history	Medical history	EBV	RPR	CMV	Hepatitis B	Hepatitis C	HIV
M	64	C	CVA	74"	150 Kg	ETOH: 2 beer/year; Tobacco: 4ppd x 15 yrs previously - quit 20 yrs ago; no drug use	Sq cell CA removed on hands 6 mos. ago, NIDDM x 10 yrs, HTN 15-20 yrs, PVD, Osteoarthritis, Bilat knee repl., kidney stones removed 33 yrs ago; Meds: Glucoside, quinine, isosorbine, metaprolol, aspirin	Not reported	Neg	Neg	Neg	Neg	Neg
M	56	C	Anoxia 2nd to Cardio-vascular	5'1"	86 Kg	Tobacco: cigarettes 2 PPD x 40 yrs, quit 1.5 yrs ago; No alcohol and drug use	Asthma, diagnosed 2 yrs ago, unk if compliant w/ treatment; COPD; Meds: Vit B & C, Albuterol inhaler, SMZ with Phenazopyridine.	Not reported	Neg	Pos	Neg	Neg	Neg
M	45	C	Anoxia; 2nd to Asphyxiation	6'2"	152lb	ETOH: (Beer, Vodka) 6-12 pack beer/day; unknown duration; occasionally drank vodka; Tobacco: 2-3 PPD, unk duration; no drug use reported.	Stomach ulcers - unknown duration or compliance. Meds: Promethazine	IgG +	Neg	Pos	Neg	Neg	Neg
M	77	C	CVA	71"	118Kg	ETOH: beer 2-3/night x 40yrs - quit 10yrs ago; Tobacco: 3ppd x 10yrs - quit 50yrs ago; no drug use	HTN x 15yrs, high cholesterol, past heart attack, diabetes 10-15 yrs, CABG 15 yrs ago, cataracts 1 - 2 yrs ago, toe surgery, renal insufficiency, CVA, TIA 10 yrs ago, allergic to penicillin, blood transfusion during CABG, arthritis in hips, fingers, spine; exposed to asbestos in past. Meds: HTN meds, Humalog, Lantis, Aranesp, ASA, Calcitrol, Lasix, Hytrin, Lipitor, Diovan, Niaspan ER, Tencimin, Coumadin, Vit. B.	Not reported	Neg	Pos	Neg	Neg	Neg
M	51	C	CVA	70"	161lb	ETOH: daily to weekly, beer moderately; Tobacco: smoker x 30 yrs; Drugs: marijuana in 80's	T2D x 1 -2 yrs-compliant, kidney stones, HTN x 10 yrs - compliant	Not reported	Neg	Neg	Neg	Neg	Neg

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Gender	Age	Race	Cause of death	Height	Weight	Social history	Medical history	EBV	RPR	CMV	Hepatitis B	Hepatitis C	HIV
M	59	AA	ICH; 2nd to CVA	6'	89 Kg	ETOH: Beer/Wine, quit > 25 years ago; Tobacco: quit > 25 years ago; Drugs: quit > 25yrs ago	HTN - unknown duration	Not reported	Not reported	Pos	Neg	Neg	Neg
M	44	C	Head Trauma/ GSW	70"	71.4Kg	ETOH: Vodka 1-2 liters, beer 12-18 (per day?) cans since young age. Heavy drinking began 12 yrs ago. Tobacco: Chew 2-3 cans per day x 31yrs; no drug use.	HTN x5yrs - non-compliant. Meds: HBP, possible pain meds	IgG+	Neg	Neg	Neg	Neg	Neg
M	38	C	Blunt Head Trauma: 2nd to MVA	74"	124Kg	ETOH: 3-5 beers socially on weekends; Tobacco: 1-2 ppw x 15 yrs; Drugs: none reported but positive for THC on Admit and benzos (after ACLS meds)	Hernia repair - 1 yr ago, eyelid laceration - 16 yrs ago, c/o stiff knees	Not reported	Neg	Neg	Neg	Neg	Neg
M	51	C	IC Bleed	70"	298lb	ETOH: as a teen; no tobacco use; Drugs: Marijuana as a teen	Diabetes Type II, HTN x 6yrs, noncaseating granuloma	Not reported	Neg	Pos	Neg	Neg	Neg
M	41	C	Head trauma; 2nd to SIGSW to the head	74"	90 Kg	ETOH: 1-2 per month; Tobacco: 2ppd x 25yrs; Drugs: Marijuana once per week.	Pero-anal abscess 1 week ago, felicitis, bronchitis 1 month ago, chronic back pain, non-cancerous lipoma.	Not reported	Neg	Pos	Neg	Neg	Neg
M	59	C	CVA	68"	218lb	No ETOH or drug use; Tobacco: 3ppd	Prostatectomy, coronary artery disease, HTN-compliant, cardiac stents, COPD & emphysema. Meds: Inhalers	Not reported	Neg	Pos	Neg	Neg	Neg
M	53	C	CVA 2nd to ICH	72 "	100Kg	ETOH: few beer/month x 4 years; no Tobacco or drug use	HTN-inconsistent compliance w/ meds (4 yrs), Type 1 IDDM - inconst. compliance w/ meds (4 yrs). Carpal Tunnel Sx 2 yrs ago, Heart Attack 4 yrs ago; Meds: HTN meds, Insulin, coumadin, over the counter prostate/urination meds.	Not reported	Neg	Neg	Neg	Neg	Neg

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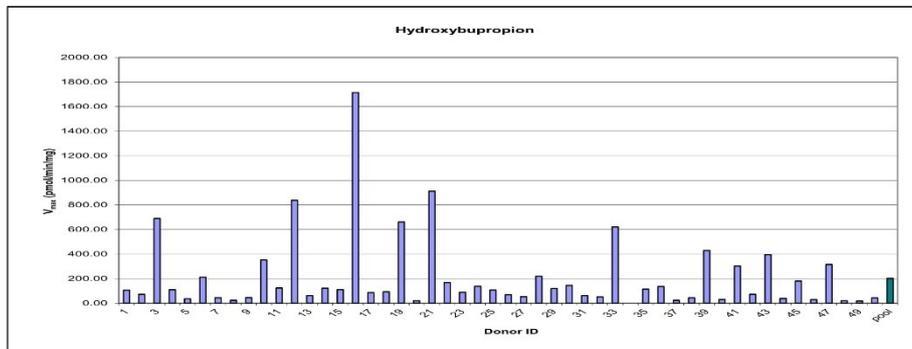
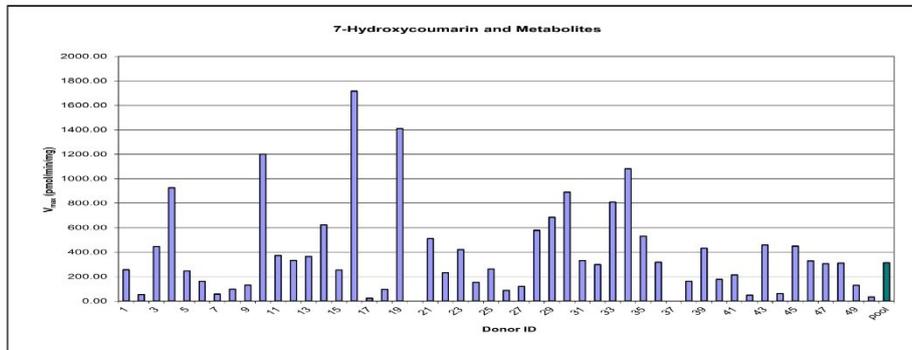
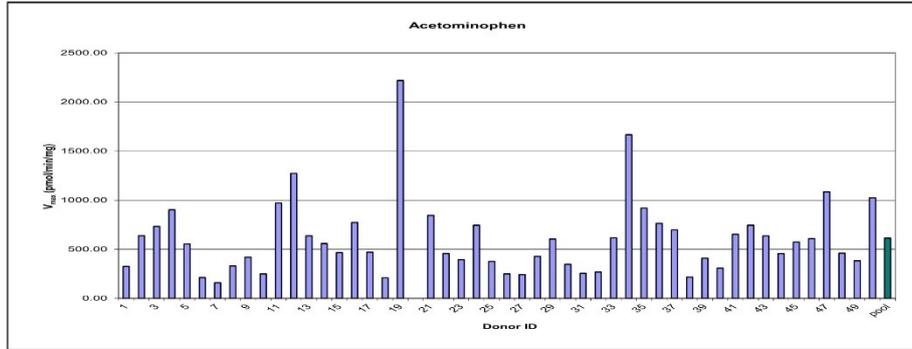
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Gender	Age	Race	Cause of death	Height	Weight	Social history	Medical history	EBV	RPR	CMV	Hepatitis B	Hepatitis C	HIV
M	78	C	Anoxia	73"	244lb	No ETOH, Tobacco or drug use	HTN x 10yrs, CAD, GI disease, black lungs.	Not reported	Neg	Pos	Neg	Neg	Neg
M	60	C	CVA/Stroke	70"	90 Kg	ETOH: Occasional in the last 5 yrs, 1 glass wine or cocktail 2x/week, also mos without drinking; no tobacco or drug use.	Partial right lung lobectomy 7 yrs ago - streptococcus, recent ear infection, flu shot, recent levoquin for possible positive blood culture. Meds: Amoxicillin	Not reported	Neg	Pos	Neg	Neg	Neg
M	45	C	CVA 2nd to ICH	5'9"	220lb	No ETOH; Tobacco: 1 -1 1/2 PPD x 28 yrs; Drugs: poss Marijuana use as per historian, unk amt, frequency, duration	HTN dx 1yr ago - unk if compliant w/ meds. Meds: Tylenol Cold/Sinus, Antacids	Neg	Neg	Neg	Neg	Neg	Neg
M	51	C	CVA; 2nd to ICH	5'10"	192 lb	ETOH: 4 cases beer per wk x19 yrs; Tobacco: 2 ppd x 30 yrs; no drug use	Coronary Artery Disease - stents placed 2 yrs ago, Hypertension x2yrs - non-compliant, recent kidney issues - dehydration due to pancreatitis, dialysis 1X earlier this year. Meds: Meds for Hypertension & Plavix - recently stopped taking meds.	IgG+	Neg	Pos	Neg	Neg	Neg
M	50	C	ICH	71"	109Kg	ETOH: Alcohol Abuse; No tobacco or drugs	HTN. Meds: none noted	Pos	Neg	Pos	Neg	Neg	Neg
M	24	C	Anoxia; 2nd to Stroke	72"	129Kg	ETOH: socially; Tobacco: 1 -2 cig occasionally; no drug use.	Valve replacement. Meds: Coumadin	Not reported	Neg	Pos	Neg	Neg	Neg
M	56	C	Head Trauma; 2nd to fell down stairs	70"	160lb	ETOH: 2-3 beers/day; Tobacco: 1ppd x 26 yrs; no drug use	High Cholesterol, High Blood Pressure, recent Bypass-Groin; Meds: Plavix, Fish Oil, Cholesterol meds	Not reported	Neg	Neg	Neg	Neg	Neg

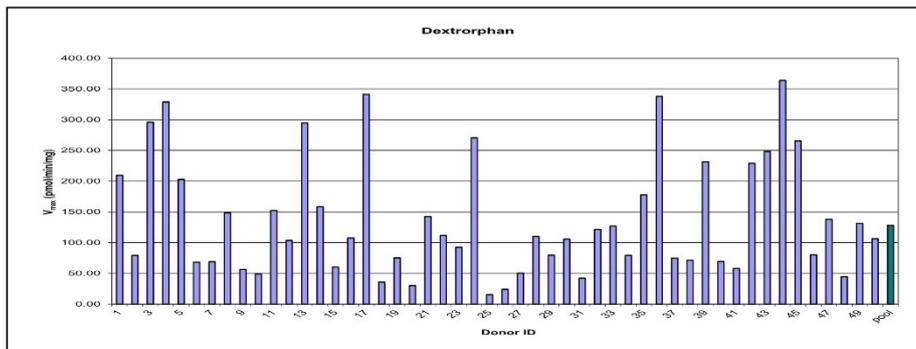
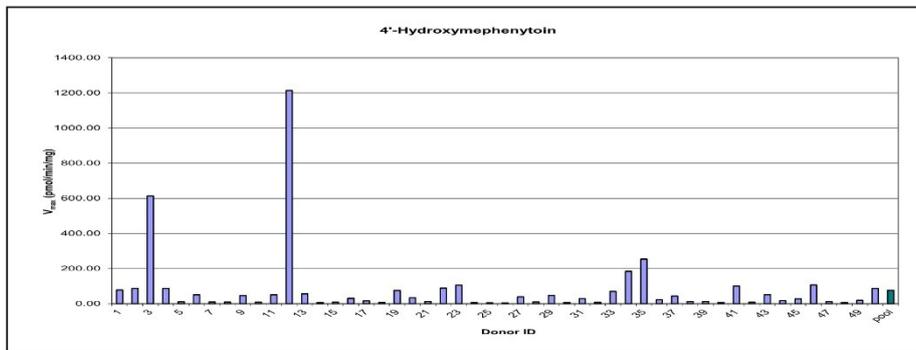
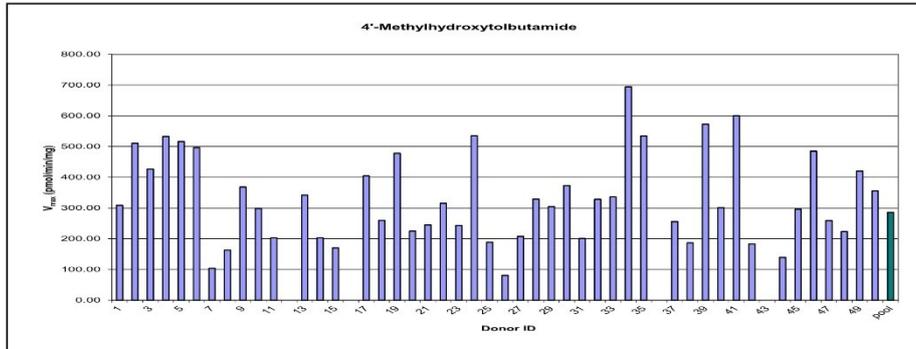
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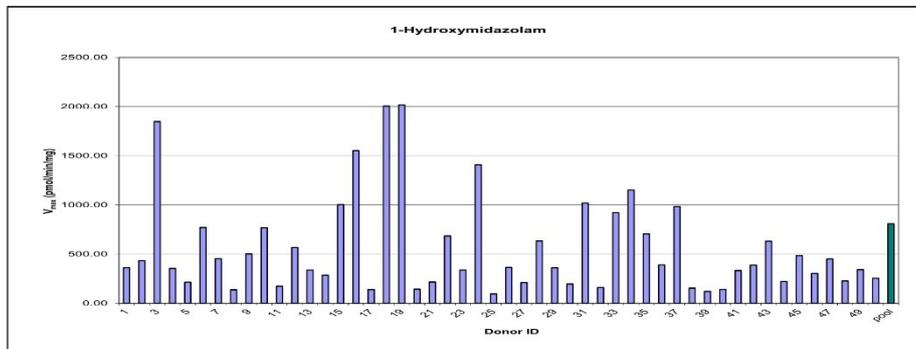
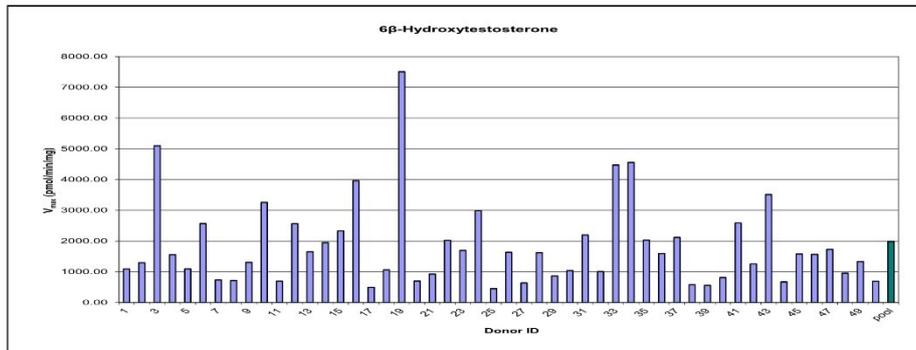
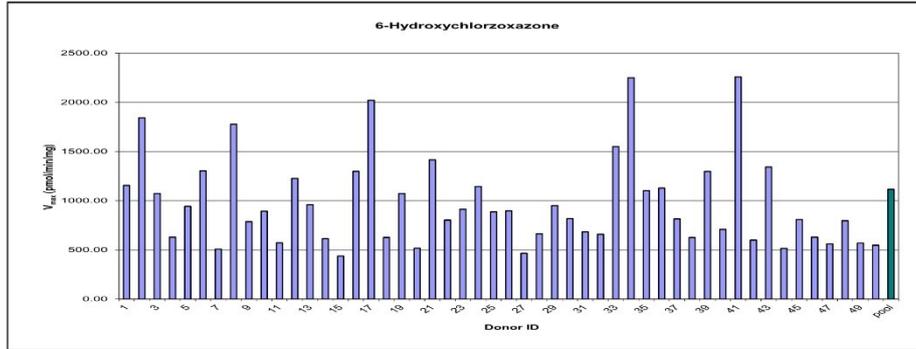
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S2 Gene test report of HepG2 cells used in this study.



Cell Line Authentication Service STR Profile Report

Sample Submitted By: Dr. Guangbo Ge
Shanghai University of Traditional Chinese Medicine
Email Address: geguangbo@dicp.ac.cn
Sales Order: 190313C
Cell Line Designation: HepG2
Date Sample Received: Mar 13th, 2019
Report Date: Mar 14th, 2019

Methodology: Nineteen short tandem repeat (STR) loci plus the gender determining locus, Amelogenin, were amplified using the commercially available EX20 Kit from AGCU. The cell line sample was processed using the ABI Prism® 3130 XL Genetic Analyzer. Data were analyzed using GeneMapper® ID v3.2 software (Applied Biosystems). Appropriate positive and negative controls were run and confirmed for each sample submitted.

Data Interpretation: Cell lines were authenticated using Short Tandem Repeat (STR) analysis as described in 2012 in ANSI Standard (ASN-0002) by the ATCC Standards Development Organization (SDO) and in Capes-Davis et al., Match criteria for human cell line authentication: Where do we draw the line? *Int J Cancer*. 2013;132(11):2510-9.

GTB™ performs STR Profiling following ISO 9001:2008 and ISO/IEC 17025:2005 quality standards.

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NOTE: According to the recommendations of *IJC* on cell line authentication, the report is valid for four years since the issue date.

Technical Questions?
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Jiangsu, P.R. China

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SIP, Suzhou, 215123
Jiangsu, P.R. China

**Cell Line Authentication Service
STR Profile Report**

Sales Order: 190313C

Test Results for Submitted Sample			DSMZ Reference Database Profile	
Loci	Query Profile: HepG2		Database Profile: HepG2	
Amelogenin	X	Y	X	Y
D3S1358	15	16		
D13S317	9	13	9	13
D7S820	10		10	
D16S539	12		12	13
Penta E	15	20		
TPOX	8	9	8	9
TH01	9		9	
D2S1338	19	20		
CSF1PO	10	11	10	11
Penta D	9	13		
D19S433	15.2			
vWA	17		17	
D21S11	29	30 31		
D18S51	13	14		
D6S1043	13			
D8S1179	15	16		
D5S818	11	12	11	12
D12S391	21	25		
FGA	22	25		

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.

Note: Loci highlighted in grey (8 core STR loci plus Amelogenin) can be made public to verify cell identity. In order to protect the identity of the donor, **please do not publish** the allele calls from all the STR loci tested.

Explanation of Test Results

Cell lines with ≥80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

- The submitted sample profile is human, but not a match for any profile in the DSMZ STR database.
- The submitted profile is an exact match for the following human cell line(s) in the DSMZ STR database (8 core loci plus Amelogenin):
- The submitted profile is similar to the following DSMZ human cell line(s): HepG2 (94% match)

e-Signature Technician:



e-Signature Reviewer:

Digitally signed by Faye Wong
DN: cn=Faye Wong, o=Genetic Testing Biotechnology (Suzhou), ou=DNA Typing Section, email=order@jsdna.org, c=CN
Date: 2019.03.14 09:51:58 +08'00'

Digitally signed by Alan Cui
DN: cn=Alan Cui, o=Genetic Testing Biotechnology (Suzhou), ou=Supervision Section, email=service@jsdna.org, c=CN
Date: 2019.03.14 09:52:34 +08'00'



More information

Addendum: Electropherogram for the customer's sample set 1 of 1



Cell Line Authentication Service STR Profile Report

