CryostaX Pellets: Improving Hepatocyte Pooling, Activity and Lab Efficiency



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XenoTech Overview

- •GLP-compliant *in vitro* ADME-DMPK CRO founded in 1994 at the University of Kansas Medical Center
- Provide services and products relevant to ADME-DMPK studies
- •In August 2008 XenoTech was acquired by Sekisui, Inc. (part of Sekisui Medical Division, Ltd)
- •In the fall of 2015, XenoTech relocated to current facility in Kansas City, KS (*pictured right*)
- •~130 employees at US facility
- •Global client base, including companies (> 300) involved in the development of pharmaceuticals, cosmetics, food additives
- •Global distributors in Europe, Japan, Korea, India, China and Singapore





• XenoTech is a provider of key reagents and test systems for *in vitro* drug metabolism studies

Subcellular Fractions

- Microsomes, S9, Cytosol
- Human and pre-clinical species
- Hepatic and extrahepatic (intestine, kidney, lung, etc.)
- Lysosomes/tritosomes

Hepatocytes

- Human
- Numerous pre-clinical species (mouse, rat, dog, monkey, etc.)
- Fresh or cryopreserved
- Attaching or suspension
- Individual or pooled

Other ADME Products

- Immortalized hepatocytes (Fa2N-4 cells)
- Multiple reagents (*e.g.* NADPH-generating system)
- Biobank
- Custom products
- We use these products in our drug metabolism services.

This enables a comprehensive understanding of the test systems that we provide to valued customers

CryostaX

CryostaX Hepatocytes

What are they?

CryostaX[™]



A BioIVT Company

CryostaX[™]

How is CryostaX going to help me and my research?

Traditional Method for Preparing Animal Primary Hepatocytes



Cryo**staX**

Method for Preparing CryostaX Animal Primary Hepatocytes

CryostaX



Simplified Product Offering

- CryostaX hepatocytes have 1 standardized <u>Assured Minimal</u> <u>Yield</u> (AMY) post thaw per species.
 - Sprague Dawley Rat = 5.0 x 10⁶ per vial
 - CD1 Mouse = 4.0 x 10^6 per vial
 - Cynomolgus Monkey = TBD

A BioIVT Company

- Rodents have a single format for use in either suspension or attaching cultures.
- Same extensive characterization as non-CryostaX hepatocytes.



Simplified Thaw Procedure

A BiolVT Company

Same Great Quality

CryostaX[®]

Recent vial lot —

R1000.H15+ Lot No. 1710151

Cryopreserved Sprague Dawley (SD) Rat Hepatocytes Male, Pool of 8

Assured Minimum Yield: Viability: 7.0 x 10^6 cells per vial 79%

Livers were perfused and subjected to collagenase digestion for the purpose of hepatocyte isolation.

Enzyme Activities		Rate
7-Ethoxycoumarin O-dealkylation	(pmol/million cells/min)	127 ± 5
7-Hydroxycoumarin glucuronidation	(pmol/million cells/min)	203 ± 12
7-Hydroxycoumarin sulfonation	(pmol/million cells/min)	90.2 ± 16.3

New CryostaX lot --->

CryostaX Single Freeze Pooled Plateable Cry	vopreserved Rat Hepatocytes	
	107	
RPCH1000+ LOT NO. 1810	J167	
Male, Pool of 16		
Assured Minimum Yield: Viability:	5.0 x 10 ⁶ cells per vial 78%	
Livers were perfused and subjected to collagenase dige	estion for the purpose of hepatocyte isolation.	
Enzyme Activities		Rate
7-Ethoxycoumarin O-dealkylation 7-Hydroxycoumarin glucuronidation 7-Hydroxycoumarin sulfonation	(pmol/million cells/min) (pmol/million cells/min) (pmol/million cells/min)	209 ± 14 216 ± 5 126 ± 4

A BiolVT Company

Less Cryoinjury in hepatocyte Pools = higher activity

Cryosta



A BiolVT Company

Cryoinjury and Effects on Phase I and II Enzymes in Pooled Human Hepatocytes

CryostaX



Freezing Effects





Single freeze pool (5 donors)

Multiple freezes pool (5 donors)

Less Cryoinjury to Pooled Human Hepatocytes

Isolate hepatocytes

Freeze and store CryostaX pellets



Only One Freeze

Cryostax

Pool one (or more) frozen pellet from each donor directly into vial

CryostaXTM Customization at no extra cost

- Quick turn around (3-4 business days)
- Examples of custom pools
 - Gender specific (male or female)
 - Specific ethnicity (i.e, Asian, Caucasian, Hispanic)
 - High or low specific enzyme activity (i.e., high CYP3A4)
 - Number of donors in pool (n=2 20)
 - Smaller or larger number of hepatocytes in vial (standard number is >5 million per vial)
 - Serology specific, such as CMV negative donors



A BiolVT Company

CryostaX[™] Customization

GP Item Number	GP Lot Number	Qty Avail For Sale	Gender	Age	Ethnicity	COD	CMV	Smoker	Alcoh	Drug Use	Diabetic	CYP1A2 ACE	CYP2A6 OCM	CYP2B6 OBP	CYP2C8 DAQ	CYP2C9 ODC	CYP2C19 40MP	CYP2D8 ODX	CYP2E1 OCZ	CYP3A4/5 6BOT	CYP3A4/ 10M
HP100	H0964	82	M	47	С	Anoxia	P	Y	Y	N	N	24	10	185	261	638	3	75	141	384	63
HP100	H0970	1397	M	68	с	Anoxia	P	N	Y	N	N	152	123	175	131	270	14	25	96	1050	256
HP100	H1023	55	M	44	С	Anoxia	P	Y	Y	N	N	11	14	48	154	79	1	23	113	445	100
HP100	H1038	60	M	13	с	Anoxia	P	N	N	N	N	38	25	28	46	62	1	14	215	155	16
HP100	H1053	33	M	5	с	HT	P	N	N	N	N	28	19	29	42	363	10	38	302	66	9
HP100	H1136	111	F	47	AA	CVA	P	Y	Y	N	N	62	101	423	341	192	36	43	33	581	165
HP100	H1155	378	F	43	С	CVA	N	N	Y	N	N	12	46	15	52	130	28	19	33	175	30
HP100	H1165	362	M	13	С	Anoxia	P	N	N	N	N	42	70	180	68	256	2	20	72	694	287
HP100	H1175	170	M	58	Α	CVA	P	N	Y	N	N	55	28	59	106	293	2	31	28	29	11
HP100	H1178	714	F	42	с	Anoxia	N	Y	Y	Y	Y	68	49	33	24	132	6	16	52	241	34
HP100	H1179	599	F	69	A	CVA	N	N	Y	N	N	21	5	10	55	87	3	31	54	60	6
HP100	H1193	242	F	57	С	Anoxia	N	Y	Y	N	N	12	27	55	72	130	1	17	44	74	14
HP100	H1211	2862	F	9	С	Anoxia	N	N	N	N	N	12	6	20	81	202	14	55	354	12	6
HP100	H1216	456	M	19	с	HT	P	Y	Y	N	N	62	62	51	75	203	12	37	106	266	91
HP100	H1218	795	F	24	с	HT	N	Y	Y	Y	Y	16	2	7	55	253	5	53	159	33	6
HP100	H1222	1246	F	56	С	Anoxia	P	N	Y	N	N	23	244	116	89	240	34	26	27	612	266
HP100	H1229	1004	M	41	AA	CVA	P	Y	N	N	N	31	45	185	208	67	1	82	22	134	82
HP100	H1246	1248	F	59	С	CVA	N	Y	Y	Y	Y	39	6	30	253	251	10	131	44	191	81
HP100	H1249	943	F	39	с	CVA	N	Y	N	N	N	16	7	16	113	102	11	17	99	132	16
HP100	H1254	1224	F	62	С	CVA	N	N	N	N	N	45	58	55	259	180	31	28	89	218	32
HP50	H1005	400	M	45	С	CVA	N	Y	N	Y	Y	114	27	80	273	458	6	49	51	291	67
HP50	H1023	50	M	44	С	Anoxia	P	Y	Y	N	N	11	14	48	154	79	1	23	113	445	100
HP50	H1026	50	F	55	AA	HT	P	Y	Y	N	N	26	32	8	54	115	1	47	49	62	35
HP50	H1038	370	M	13	с	Anoxia	P	N	N	N	N	38	25	28	46	62	1	14	215	155	16
HP50	H1053	753	M	5	С	HT	P	N	N	N	N	28	19	29	42	363	10	38	302	66	9
HP50	H1081	450	M	55	С	CVA	P	Y	Y	N	N	33	14	21	94	226	1	42	237	34	8
HP50	H1092	120	F	9	A	Anoxia	N	N	N	N	N	22	10	14	225	467	20	41	145	263	35
HP50	H1109	150	F	71	С	CVA	N	Y	Y	N	N	288	46	54	213	415	2	51	109	485	39
HP50	H1136	600	F	47	AA	CVA	P	Y	Y	N	N	62	101	423	341	192	36	43	33	581	165
HP50	H1146	410	F	48	С	CVA	N	Y	N	N	N	88	26	14	221	250	33	33	125	304	35
HP50	H1149	490	F	31	С	Anoxia	N	Y	Y	Y	Y	213	62	171	345	410	386	132	504	1060	228
HP50	H1152	610	F	69	A	CVA	P	N	N	N	N	8	24	22	171	199	3	24	197	94	48
HP50	H1155	570	F	43	С	CVA	N	N	Y	N	N	12	46	15	52	130	28	19	33	175	30
HP50	H1190	440	F	71	С	CVA	P	N	Y	N	N	41	14	32	41	202	5	40	65	119	17
HP50	H1193	290	F	57	С	Anoxia	Ν	Y	Y	N	N	12	27	55	72	130	1	17	44	74	14
HP50	H1223	860	М	47	С	Anoxia	N	N	N	N	N	94	49	45	81	204	4	40	95	323	124
HP50	H1226	690	F	63	1	Anoxia	P	N	Y	N	N	48	37	23	139	280	5	37	139	336	105

Hepatocytes from each donor are fully characterized for CYP activities

EXPERTISE • EFFICIENCY • SUPPORT • PRECISION

CryostaX[®]

CryostaX[™] Customization

Theoretical Activity Data vs. Actual Activity Data

Cryo**staX**



CryostaXTM Pooled Plateable Hepatocytes

	Activity (pmol/min/10 ⁶ cells)								
Hepatocyte Lot	1A2	2B6	2C8	2C9	2C19	2D6	3A4	SULT	UGT
Donor A	1.8	1.7	16	0.5	0.3	3.9	1.1	2.0	92
Donor B	3.1	1.7	102	1.3	2.9	5.7	9.0	4.2	159
Donor C	12	2.3	77	0.6	0.1	3.3	4.9	2.8	121
Donor D	5.5	13	390	3.5	2.1	18	16	19	600
Donor E	17	4.4	227	2.3	6.6	14	16	7.1	212
Individual Avg	8.0	4.7	162	1.6	2.4	8.9	9.4	7.0	237
Pooled	7.3	5.4	198	1.4	1.5	9.0	10.8	7.3	253

Enzyme activity rates of the pool closely match the average enzyme activity rates of all individuals in the pool, suggesting that each donor is well represented and that one donor does not over-contribute or under-contribute.

CryostaXTM Pooled Plateable Hepatocytes



CYP1A2, 2B6, 2C8, 2C9, 2C19 and 3A4 enzyme activity and mRNA were all inducible by prototypical inducers

CryostaXTM Pooled Plateable Hepatocytes

Cl_{int} values for 14 compounds with CryostaX pooled plateable hepatocytes



CryostaX[™] Pooled Plateable Hepatocytes



Good agreement with other currently available test systems for low turn over drug clearance assessment

Crvo**staX**

Pooled plateable hepatocytes	Micro-patterned hepatocytes	Hepatocyte relay method
Cost effective and easy to use	Cost prohibitive	Labor intensive

CryostaX[™] Pooled Hepatocytes

CryostaX™ Pooled Human Hepatocytes

- Available in suspension or plateable formats.
- Available as individual donors with custom AMY.
- The only available human pooled hepatocytes subjected to only ONE cryopreservation process.
- Easily custom assembled according to customer specified criteria activity, ethnicity, demographics, genotype
- Effective for evaluating low, mid, and high clearance drugs
- CryostaX[™] Plateable Hepatocytes:
 - suitable for hepatic uptake studies.
 - can be utilized for CYP induction screening.
- Simple thaw and utilization procedure

Opti^{Media}

Product ID	Product Name	Volume	Product Details
K8000		47 mL	Cell culture media optimized for thawing cryopreserved hepatocytes, plus Trypan blue solution to obtain accurate cell yield and viability.
K8100	Opti THAW Hepatocyte Kit	45 mL	Cell culture media optimized for thawing cryopreserved mouse hepatocytes plus Trypan blue solution to obtain accurate cell yield and viability.
K8200		40 mL	Cell culture media optimized for plating hepatocytes.
K8300	Op†i CULTURE Hepatocyte Media	100 mL	Cell culture media optimized for culturing plated hepatocytes. This is a serum-free media. The Pen/Strep supplement is packaged separately for addition when ready to use the media.
K8400	Op†i INCUBATE Hepatocyte Media	50 mL	Culture media optimized for incubation of hepatocytes in suspension.
K8500	SHEPATOSURE Opti thaw	47 mL	Cell culture media optimized for thawing Hepatosure™ Pooled cryopreserved hepatocytes, plus Trypan blue solution to obtain accurate cell yield and viability.
K8700		100 mL	Media for the thawing and culture of Kupffer cells.

CryostaX

Improved Attachment Efficiency Improved Confluency and Morphology **Extended Expiration dates**

CryostaX™

Customizable, single-freeze pooled hepatocytes (n = 2 to 20) or individual donors



Customizable, PLATEABLE single-freeze pooled hepatocytes (n = 2 to 20) or individual donors

Geneknown Human Hepatocytes

Pooled genotyped human hepatocytes





Animal hepatocytes -Sprague Dawley Rat CD-1 Mouse Cynomolgus Monkey (soon!)



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- Hepatocytes (Cryo/Fresh, Genotyped...)
- Non-Parenchymal Cells (Kupffer Cells)

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- Liver Microsomes
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- Homogenate
- Lysosomes & Tritosomes
- Mitochondria
 Extrahepatic Fractions

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Various Species, Tissues & Preparations

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