

Expi293 Expression System

Boosting protein expression to >1 g/L in a HEK 293 mammalian transient protein production system

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Abstract

Transient expression provides a rapid, flexible, and economical protein production alternative to the time-consuming and costly process of generating and selecting stable, high-expressing cell lines. However, there is a growing demand for the higher protein yields necessary to feed the increasing needs of pharmaceutical research. This study shows how redesigning the transient expression model enables the superior protein yield and reproducibility typically obtained with stable cell lines while maintaining the simplicity and ease of use associated with current transient expression systems. The combination of a unique high-capacity medium, a 293F cell variant, and the Gibco™ ExpiFectamine 293™ transfection reagent and enhancer together enable the Gibco™ Expi293™ Expression System to achieve expression levels approaching and exceeding 1 g/L of protein within 7 days. We present data demonstrating the ability of the medium to support the increased growth rate, transfection efficiency, and higher levels of protein productivity of the Gibco™ Expi293F™ cell line. In some cases, expression of both IgG and non-IgG proteins was increased up to 12-fold over levels obtained with the Invitrogen™ FreeStyle™ 293 Expression System. Proteins produced in the Expi293 system exhibit biologically relevant target recognition, binding, and native pharmacokinetic performance. The system is scalable and, as a result of the high yields, enables the use of microplates for high-throughput applications. The system has been demonstrated to maintain high yields with a wide variety of proteins and applications, including lentiviral and membrane protein production. By enabling the generation of substantially higher levels of protein in smaller culture volumes, the Expi293 system represents a powerful, economical, and unique expression technology for both research and production of therapeutic candidate proteins.

The system

Product	Quantity	Cat. No.
Expi293 Expression Medium	1,000 mL	A1435101
Expi293 Expression Medium	6 x 1 L	A1435102
ExpiFectamine 293 Transfection Kit	For 1 L culture	A14524
ExpiFectamine 293 Transfection Kit	For 10 L culture	A14525
ExpiFectamine 293 Transfection Kit	For 50 L culture	A14526
Expi293F Cells	1 mL	A14527
Expi293F Cells	6 x 1 mL	A14528
Expi293 Expression System Kit	1 kit	A14635
Antibody-Expressing Positive Control Vector	1 vial	A14662
pcDNA 3.4 TOPO TA Cloning Kit	1 kit	A14697



Results

Unique high-density medium

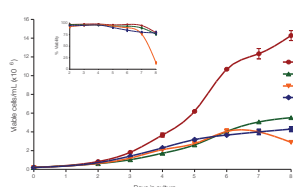


Figure 1. Expi293F cells were adapted into various media over three passages. Cells were cultured for multiple passages in each medium before being seeded at 0.2×10^6 cells/mL. Cell density and viability were monitored over 8 days. Gibco™ Expi293™ Expression Medium is unique in its support of high cell densities while still supporting transfections (see Figure 3).

Cell line optimization

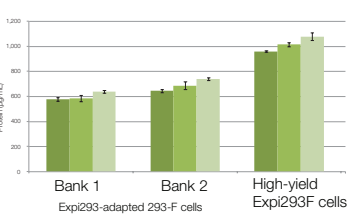


Figure 2. A subpopulation of Invitrogen™ FreeStyle™ 293-F Cells adapted into Expi293 medium demonstrated greater viable cell densities, average diameter, and up to 50% higher protein expression capacity than FreeStyle 293-F cells.

Enhancers boost expression with ExpiFectamine 293 Reagent

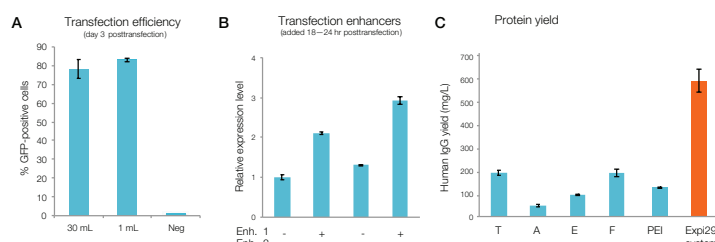


Figure 3. (A) ExpiFectamine 293 Reagent achieves high-efficiency transfection of high-density cultures. (B) A combination of enhancers boosts protein yield almost 3-fold. (C) Enhancers work specifically with ExpiFectamine 293 Reagent to achieve high protein yields.

Up to 12x yields compared to FreeStyle system, at >1 g protein/L

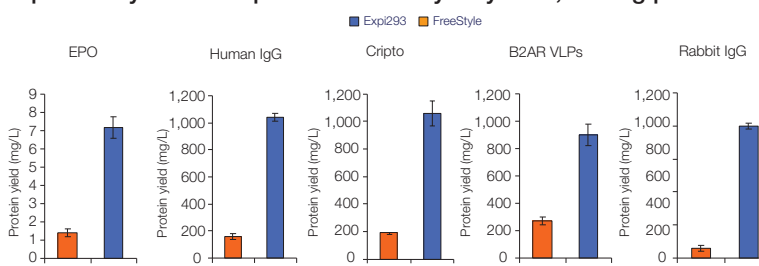


Figure 4. Expression levels of five proteins demonstrate a 3–12x increase in protein expression in the Expi293 system relative to the FreeStyle 293 system. Expression levels exceeded 1 g/L for a secreted antibody (human IgG) and a cell surface receptor (Cripto).

Reproducibility and scalability

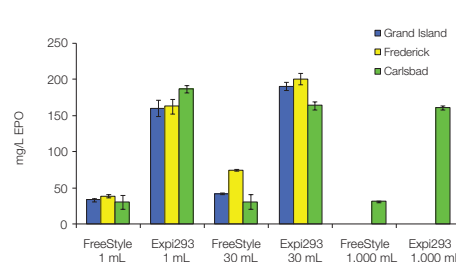


Figure 5. Protein yield per unit volume remains constant from 1 mL (24-well plate) to 30 mL (flask) to 1,000 mL (3 L Fernbach). Expression in 96-well format and preliminary work in 10 L WAVE™ bags also demonstrate excellent scalability (not shown).

EPO was expressed in three culture formats at labs in Grand Island, Frederick, and Carlsbad. Despite differences in operator handling and culture facilities, EPO yield variability in the Expi293 system was within 20% across all formats.

Protein quality and functionality are maintained

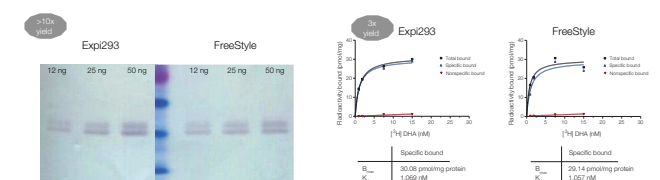


Figure 6. Antibodies produced in the Expi293 and FreeStyle systems show equivalent antigen reactivity.

Figure 7. The G protein-coupled receptor (GPCR) B2AR demonstrates comparable and physiologically specific antagonist binding whether produced in the Expi293 system or the FreeStyle system.

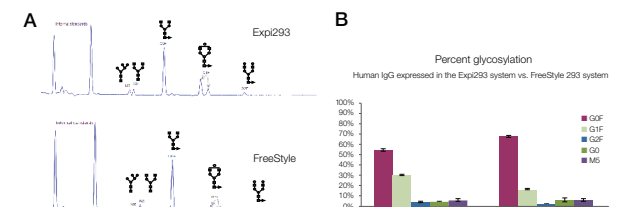
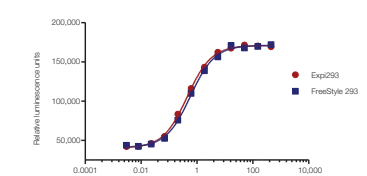


Figure 8. (A) Antibodies produced in both expression systems show similar patterns of glycosylation. (B) The preparations are also similar in the extent to which each glycosylation moiety is present on the expressed protein.

Figure 9. EPO made in the Expi293 and FreeStyle 293 systems was tested in a TF-1 cell proliferation assay. Both preparations showed equivalent stimulatory activity.



Alternative applications for the Expi293 system

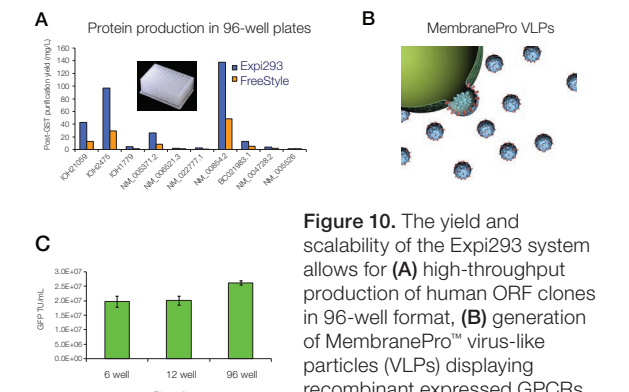


Figure 10. The yield and scalability of the Expi293 system allows for (A) high-throughput production of human ORF clones in 96-well format, (B) generation of MembranePro™ virus-like particles (VLPs) displaying recombinant expressed GPCRs using the Expi293 system (see Figure 7 above), and (C) high-titer production of lentivirus.

Conclusions

The Expi293 system defines the next generation of protein production in mammalian cells, producing more protein in less culture volume than any other commercial mammalian system. By doing so, it offers better economy in the use of equipment, tissue culture capacity, plasmid DNA, and plastic cultureware. The high yield, linear scalability, and versatility of the system enables the use of microplates for high-throughput applications and offers a simple and high-efficiency method for the production of lentivirus and MembranePro particles. As a complete system that is simple and easy to use, the Expi293 system offers the leading solution for mammalian protein expression.

“In all my years working with transient expression systems, the Expi293 Expression System is the first one to achieve 2.3 g/L, beating every other HEK 293 transient expression system.”

Jelte-Jan Reitsma, research associate

Definition	Gene symbol	Protein	Expi293 yield (µg/mL)
Pleckstrin, mRNA (cDNA clone MGC:17111 IMAGE:4341823), complete cds	PLEK	AAH18549.1	5,610
Signal transducer and activator of transcription 3 (acute-phase response factor) (STAT3), transcript variant 1, mRNA	STAT3	NP_644805.1	2,139
BH3 interacting domain death agonist (BID), transcript variant 2, mRNA	BID	NP_001187.1	972
RAB38, member RAS oncogene family (RAB38), mRNA	RAB38	NP_071732.1	702
S100 calcium binding protein A4 (S100A4), transcript variant 1, mRNA	S100A4	NP_002952.1	609
NAD(P)H dehydrogenase, quinone 1 (NQO1), transcript variant 1, mRNA	NQO1	NP_000894.1	525
Prosaposin (PSAP), transcript variant 1, mRNA	PSAP	NP_002769.1	524
Transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha) (TFAP2A), transcript variant 2, mRNA	TFAP2A	NP_001027451.1	478
TH1-like (Drosophila), mRNA (cDNA clone MGC:22971 IMAGE:4860894), complete cds	NELFCD	AAH14952.1	411
Growth factor receptor-bound protein 2 (GRB2), transcript variant 1, mRNA	GRB2	NP_002077.1	333
Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein (ID1), transcript variant 1, mRNA	ID1	NP_002156.2	295
S100 calcium binding protein A6 (S100A6), mRNA	S100A6	NP_055439.1	246
Epidermal growth factor receptor (EGFR), transcript variant 3, mRNA	EGFR	NP_958440.1	206
Integrin-linked kinase (ILK), transcript variant 2, mRNA	ILK	NP_001014794.1	199
S100 calcium binding protein P (S100P), mRNA	S100P	NP_005971.1	197
Methylthioadenosine phosphorylase, mRNA (cDNA clone MGC:33067 IMAGE:4820938), complete cds	MTAP	AAH26106.1	182
Ornithine aminotransferase (OAT), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	OAT	NP_000265.1	179
Chromosome 12 open reading frame 57 (C12orf57), mRNA	C12orf57	NP_612434.1	175
Tumor suppressor candidate 4, mRNA (cDNA clone MGC:22898 IMAGE:4068981), complete cds	NPRL2	AAH21984.1	175
N-myc downstream regulated 1 (NDRG1), transcript variant 2, mRNA	NDRG1	NP_006087.2	162
MutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) (MSH2), transcript variant 1, mRNA	MSH2	NP_000242.1	159
Cairitculin (CALR), mRNA	CALR	NP_004334.1	148
Major vault protein (MVP), transcript variant 2, mRNA	MVP	NP_005106.2	139
Mitogen-activated protein kinase kinase 4, mRNA (cDNA clone MGC:33126 IMAGE:5272439), complete cds	MAP2K4	AAH36032.1	138
X-ray repair complementing defective repair in Chinese hamster cells 3, mRNA (cDNA clone MGC:19630 IMAGE:4138588), complete cds	XRCC3	AAH11725.1	138
Budding uninhibited by benzimidazoles 1 homolog beta (yeast), mRNA (cDNA clone MGC:31884 IMAGE:4649881), complete cds	BUB1B	AAH18739.1	135
GNAS complex locus (GNAS), transcript variant 3, mRNA	GNAS	NP_536351.1	125
CD4 molecule (CD4), transcript variant 1, mRNA	CD4	NP_000607.1	108
Tumor protein p53, mRNA (cDNA clone MGC:646 IMAGE:3544714), complete cds	TP53	AAH03596.1	100
HNF1 homeobox A (HNF1A), mRNA	HNF1A	NP_000536.5	84
Sulfotransferase family 1E, estrogen-preferring, member 1, mRNA (cDNA clone MGC:34459 IMAGE:5210178), complete cds	SULT1E1	AAH27956.1	79
Chitinase 3-like 1 (cartilage glycoprotein-39), mRNA (cDNA clone MGC:17199 IMAGE:4212419), complete cds	CHI3L1	AAH38354.1	76
C-src tyrosine kinase (CSK), transcript variant 1, mRNA	CSK	NP_004374.1	72
Thrombomodulin, mRNA (cDNA clone MGC:45302 IMAGE:5176531), complete cds	THBD	AAH35602.2	71
Janus kinase 2 (JAK2), mRNA	JAK2	NP_004963.1	69
Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B, mRNA (cDNA clone MGC:18122 IMAGE:4153377), complete cds	SEMA3B	AAH09113.1	67
HORMA domain containing 1 (HORMAD1), transcript variant 1, mRNA	HORMAD1	NP_115508.2	66
Transmembrane protein 37, mRNA (cDNA clone MGC:50757 IMAGE:5221396), complete cds	TMEM37	AAH46362.1	62
Actin-like 8, mRNA (cDNA clone MGC:24924 IMAGE:4933032), complete cds	ACTL8	AAH28909.1	56
Intercellular adhesion molecule 1 (ICAM1), mRNA	ICAM1	NP_000192.2	54
Dihydrofolate reductase, mRNA (cDNA clone MGC:88273 IMAGE:6714220), complete cds	DHFR	AAH70280.1	47
FEV (ETS oncogene family) (FEV), mRNA	FEV	NP_059991.1	47
Chromosome 8 open reading frame 4 (C8orf4), mRNA	C8orf4	NP_064515.1	46
Fatty acid binding protein 1, liver (FABP1), mRNA	FABP1	NP_001434.1	43
Matrix metalloproteinase 10 (stromelysin 2) (MMP10), mRNA	MMP10	NP_002416.1	41
Protein tyrosine phosphatase, non-receptor type 11 (PTPN11), transcript variant 2, mRNA	PTPN11	NP_542168.1	40
Olfactory receptor, family 51, subfamily E, member 2 (OR51E2), mRNA	OR51E2	NP_110401.1	39
F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase (FBXW7), transcript variant 2, mRNA	FBXW7	NP_060785.2	39
SMAD family member 4 (SMAD4), mRNA	SMAD4	NP_005350.1	35
Echinoderm microtubule associated protein like 4, mRNA (cDNA clone IMAGE:3868340), complete cds	EML4	AAH08685	33
Synovial sarcoma, X breakpoint 2 (SSX2), transcript variant 2, mRNA	SSX2	NP_783629.1	32
V-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian) (MYCN), mRNA	MYCN	NP_005369.2	32
Estrogen receptor 2 (ER beta), mRNA (cDNA clone MGC:26496 IMAGE:4825937), complete cds	ESR2	AAH24181.1	32
Cancer susceptibility candidate 1 (CASC1), transcript variant 3, mRNA	CASC1	NP_001076441.1	31
Thioredoxin (TXN), transcript variant 1, mRNA	TXN	NP_003320.2	30
Glutathione S-transferase mu 1 (GSTM1), transcript variant 2, mRNA	GSTM1	NP_666533.1	30
Fanconi anemia, complementation group C (FANCC), transcript variant 1, mRNA	FANCC	NP_000127.2	29
Cytochrome P450, family 2, subfamily A, polypeptide 6, mRNA (cDNA clone MGC:116920 IMAGE:40006064), complete cds	CYP2A6	AAH96255.1	29
Tumor necrosis factor receptor superfamily, member 10b, mRNA (cDNA clone MGC:5144 IMAGE:3458466), complete cds	TNFRSF10B	AAH01281.1	28
Peptidylprolyl cis/trans isomerase, NIMA-interacting 1 (PIN1), transcript variant 1, mRNA	PIN1	NP_006212.1	28
Placenta-specific 1 (PLAC1), mRNA	PLAC1	NP_068568.1	28
Cyclin-dependent kinase 4 (CDK4), mRNA	CDK4	NP_000066.1	28
CUB domain containing protein 1 (CDCP1), transcript variant 2, mRNA	CDCP1	NP_835488.1	27
Dihydroliipoamide dehydrogenase (DLD), nuclear gene encoding mitochondrial protein, mRNA	DLD	NP_000099.2	27
X antigen family, member 1A (XAGE1A), transcript variant a, mRNA	XAGE1A	NP_001091061.2	27
Signal transducer and activator of transcription 5A (STAT5A), mRNA	STAT5A	NP_003143.2	26
Ras homolog family member C (RHOC), transcript variant 1, mRNA	RHOC	NP_786886.1	25
Neuroblastoma RAS viral (v-ras) oncogene homolog (NRAS), mRNA	NRAS	NP_002515.1	25
Apolipoprotein D (APOD), mRNA	APOD	NP_001638.1	24
Alpha-fetoprotein (AFP), mRNA	AFP	NP_001125.1	23
BCL2-associated agonist of cell death (BAD), transcript variant 1, mRNA	BAD	NP_004313.1	22
Kallikrein-related peptidase 4, mRNA (cDNA clone MGC:96926 IMAGE:7262135), complete cds	KLK4	AAH69429.1	22
Transmembrane protease, serine 2 (TMPRSS2), transcript variant 2, mRNA	TMPRSS2	NP_005647.3	19
V-rel reticuloendotheliosis viral oncogene homolog B (RELB), mRNA	RELB	NP_006500.2	19
PDZ and LIM domain 5 (PDLIM5), transcript variant 5, mRNA	PDLIM5	NP_001011516.1	18
Diablo, IAP-binding mitochondrial protein (DIABLO), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	DIABLO	NP_063940.1	18
Chromosome X open reading frame 48 (CXorf48), transcript variant 2, mRNA	CXorf48	NP_060333.1	18
Cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA	CYR61	NP_001545.2	17
Major histocompatibility complex, class I, E, mRNA (cDNA clone MGC:1408 IMAGE:3140835), complete cds	HLA-E	AAH02578.1	17
High mobility group AT-hook 1 (HMGA1), transcript variant 2, mRNA	HMGA1	NP_002122.1	17
Ret proto-oncogene, mRNA (cDNA clone IMAGE:3507569), complete cds	RET	AAH03072	17
Angiotensin II receptor, type 2 (AGTR2), mRNA	AGTR2	NP_000677.2	17
Parathyroid hormone (PTH), mRNA	PTH	NP_000306.1	17
Transforming growth factor, beta 1, mRNA (cDNA clone MGC:2323 IMAGE:3356605), complete cds	TGFB1	AAH01180.1	16
Insulin-like growth factor binding protein 2, 36kDa (IGFBP2), mRNA	IGFBP2	NP_000588.2	16
Kallikrein-related peptidase 3 (KLK3), transcript variant 1, mRNA	KLK3	NP_001639.1	16

Definition	Gene symbol	Protein	Expi293 yield (µg/mL)
Janus kinase 3 (JAK3), mRNA	JAK3	NP_000206.2	16
Tyrosine kinase 2, mRNA (cDNA clone MGC:20776 IMAGE:4591726), complete cds	TYK2	AAH14243.1	15
BCL2-like 2 (BCL2L2), transcript variant 1, mRNA	BCL2L2	NP_004041.1	15
Keratin 5, mRNA (cDNA clone MGC:39301 IMAGE:5428482), complete cds	KRT5	AAH24292.1	15
Smoothed, frizzled family 17 receptor (SMO), mRNA	SMO	NP_005622.1	15
Cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1), mRNA	CYP17A1	NP_000093.1	15
Metallothionein 3 (MT3), mRNA	MT3	NP_005945.1	14
Nucleolin (NCL), mRNA	NCL	NP_005372.2	14
Adenosylmethionine decarboxylase 1 (AMD1), transcript variant 1, mRNA	AMD1	NP_001625.2	13
Keratin 19, mRNA (cDNA clone MGC:15788 IMAGE:3504206), complete cds	KRT19	AAH07628.1	13
Lysosomal-associated membrane protein 1 (LAMP1), mRNA	LAMP1	NP_005552.3	12
Lamin B receptor (LBR), transcript variant 1, mRNA	LBR	NP_002287.2	12
Epoxide hydrolase 1, microsomal (xenobiotic) (EPHX1), transcript variant 1, mRNA	EPHX1	NP_000111.1	12
G antigen 12l (GAGE12l), mRNA	GAGE12l	NP_001468.1	12
Prolactin receptor (PRLR), transcript variant 1, mRNA	PRLR	NP_000940.1	12
Major histocompatibility complex, class I, A, mRNA (cDNA clone MGC:17191 IMAGE:4157200), complete cds	HLA-A	AAH08611.1	12
Estrogen receptor 1 (ESR1), transcript variant 1, mRNA	ESR1	NP_000116.2	11
Twist basic helix-loop-helix transcription factor 1 (TWIST1), mRNA	TWIST1	NP_000465.1	10
ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B) (ELAVL2), transcript variant 2, mRNA	ELAVL2	NP_001164666.1	10
Ring finger protein 14 (RNF14), transcript variant 2, mRNA	RNF14	NP_899645.1	10
Synovial sarcoma translocation, chromosome 18, mRNA (cDNA clone MGC:116875 IMAGE:40004909), complete cds	SS18	AAH96222.1	10
Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) (CEACAM1), transcript variant 6, mRNA	CEACAM1	NP_001192273.1	9
Surfactant protein C, mRNA (cDNA clone MGC:14509 IMAGE:4043169), complete cds	SFTPC	AAH05913.1	9
Mucosa associated lymphoid tissue lymphoma translocation gene 1 (MALT1), transcript variant 2, mRNA	MALT1	NP_776216.1	9
Cancer/testis antigen family 45, member A1 (CT45A1), mRNA	CT45A1	NP_001017417.1	9
Fatty acid synthase, mRNA (cDNA clone IMAGE:4299348), complete cds	FASN	AAH07909	9
Oxidized low density lipoprotein (lectin-like) receptor 1 (OLR1), transcript variant 1, mRNA	OLR1	NP_002534.1	8
Fas ligand (TNF superfamily, member 6) (FASLG), mRNA	FASLG	NP_000630.1	8
Growth differentiation factor 15 (GDF15), mRNA	GDF15	NP_004855.2	8
BCL2-like 1 (BCL2L1), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	BCL2L1	NP_612815.1	8
Caveolin 1, caveolae protein, 22kDa (CAV1), transcript variant 1, mRNA	CAV1	NP_001744.2	8
Proprotein convertase subtilisin/kexin type 7, mRNA (cDNA clone MGC:12699 IMAGE:4125594), complete cds	PCSK7	AAH06357.1	8
FYN oncogene related to SRC, FGR, YES (FYN), transcript variant 1, mRNA	FYN	NP_002028.1	7
Eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1), mRNA	EIF4EBP1	NP_004086.1	7
Interleukin 10 (IL10), mRNA	IL10	NP_000563.1	7
Retinoic acid receptor, beta (RARβ), transcript variant 1, mRNA	RARB	NP_000956.2	6
Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1) (RAC1), transcript variant Rac1, mRNA	RAC1	NP_008839.2	6
Small nuclear ribonucleoprotein polypeptide E (SNRPE), mRNA	SNRPE	NP_003085.1	6
Nescient helix loop helix 1 (NHLH1), mRNA	NHLH1	NP_005589.1	6
Alpha-methylacyl-CoA racemase, mRNA (cDNA clone MGC:3743 IMAGE:2958112), complete cds	AMACR	AAH09471.1	6
Surfactant protein D, mRNA (cDNA clone MGC:22626 IMAGE:4696173), complete cds	SFTPD	AAH22318.1	6
Kallikrein-related peptidase 10, mRNA (cDNA clone MGC:3667 IMAGE:3632557), complete cds	KLK10	AAH02710.1	5
Jun proto-oncogene (JUN), mRNA	JUN	NP_002219.1	5
FEZ family zinc finger 2, mRNA (cDNA clone MGC:26179 IMAGE:4794121), complete cds	FEZF2	AAH22464.1	5
Interleukin 6 (interferon, beta 2) (IL6), mRNA	IL6	NP_000591.1	5
Lymphoid enhancer-binding factor 1 (LEF1), transcript variant 1, mRNA	LEF1	NP_057353.1	5
Cholinergic receptor, nicotinic, alpha 3 (neuronal) (CHRNA3), transcript variant 1, mRNA	CHRNA3	NP_000734.2	5
Transforming growth factor, beta receptor II (70/80kDa) (TGFB2), transcript variant 2, mRNA	TGFB2	NP_003233.4	4
Cell division cycle 25B (CDC25B), transcript variant 1, mRNA	CDC25B	NP_068659.1	4
Carboxypeptidase M (CPM), transcript variant 3, mRNA	CPM	NP_001005502.1	4
Cytochrome P450, family 3, subfamily A, polypeptide 5 (CYP3A5), transcript variant 1, mRNA	CYP3A5	NP_000768.1	4
Trefoil factor 1 (TFF1), mRNA	TFF1	NP_003216.1	4
E74-like factor 3 (ets domain transcription factor, epithelial-specific) (ELF3), transcript variant 2, mRNA	ELF3	NP_001107781.1	4
ATP-binding cassette, sub-family C (CFTR/MRP), member 5 (ABCC5), transcript variant 2, mRNA	ABCC5	NP_001018881.1	4
Keratin 18 (KRT18), transcript variant 1, mRNA	KRT18	NP_000215.1	4
Ewing sarcoma breakpoint region 1 (EWSR1), transcript variant 2, mRNA	EWSR1	NP_005234.1	4
24-dehydrocholesterol reductase (DHCR24), mRNA	DHCR24	NP_055577.1	4
Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5, mRNA (cDNA clone MGC:16086 IMAGE:3618167), complete cds	SERPINA5	AAH08915.1	4
Siah E3 ubiquitin protein ligase 1 (SIAH1), transcript variant 2, mRNA	SIAH1	NP_001006611.1	3
Pleiotrophin (PTN), mRNA	PTN	NP_002816.1	3
Frizzled-related protein (FRZB), mRNA	FRZB	NP_001454.2	3
Ribosomal protein S6 (RPS6), mRNA	RPS6	NP_001001.2	3
Folate hydrolase (prostate-specific membrane antigen) 1 (FOLH1), transcript variant 2, mRNA	FOLH1	NP_001014986.1	3
Melanoma antigen family C, 2, mRNA (cDNA clone MGC:13377 IMAGE:4133159), complete cds	MAGEC2	AAH13318.1	3
Synovial sarcoma, X breakpoint 1 (SSX1), mRNA	SSX1	NP_005626.1	3
Wingless-type MMTV integration site family, member 5A (WNT5A), transcript variant 1, mRNA	WNT5A	NP_003383.2	2
Cholecystokinin (CCK), transcript variant 1, mRNA	CCK	NP_000720.1	2
Vimentin (VIM), mRNA	VIM	NP_003371.2	2
Xeroderma pigmentosum, complementation group A (XPA), transcript variant 1, mRNA	XPA	NP_000371.1	2
Neuregulin 1, mRNA (cDNA clone IMAGE:3161700), complete cds	NRG1	AAH07675	2
Homeobox B13 (HOXB13), mRNA	HOXB13	NP_006352.2	2
Desmin (DES), mRNA	DES	NP_001918.3	2
BCL2-antagonist/killer 1 (BAK1), mRNA	BAK1	NP_001179.1	2
ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D), mRNA (cDNA clone MGC:33705 IMAGE:5286347), complete cds	ELAVL4	AAH36071.1	2
CLPTM1-like (CLPTM1L), mRNA	CLPTM1L	NP_110409.2	2
Lymphocyte-specific protein tyrosine kinase, mRNA (cDNA clone MGC:17196 IMAGE:4341278), complete cds	LCK	AAH13200.1	1
Prolactin (PRL), transcript variant 1, mRNA	PRL	NP_000939.1	1
Matrix metalloproteinase 3 (stromelysin 1, progelatinase) (MMP3), mRNA	MMP3	NP_002413.1	1
TIMP metalloproteinase inhibitor 3 (TIMP3), mRNA	TIMP3	NP_000353.1	1
Dihydroliipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex) (DLST), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA	DLST	NP_001924.2	1
Insulin-like growth factor binding protein 3, mRNA (cDNA clone MGC:2305 IMAGE:3506666), complete cds	IGFBP3	AAH00013.1	1
Matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase), mRNA (cDNA clone MGC:12688 IMAGE:4054882), complete cds	MMP9	AAH06093.1	1
Insulin-like growth factor binding protein 1, mRNA (cDNA clone MGC:30041 IMAGE:4800940), complete cds	IGFBP1	AAH35263.2	1
PAS domain containing 1 (PASD1), mRNA	PASD1	NP_775764.2	1
Interleukin 8 (IL8), mRNA	IL8	NP_000575.1	1
Melanoma inhibitory activity (MIA), transcript variant 1, mRNA	MIA	NP_006524.1	1

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