

Diethylstilbestrol

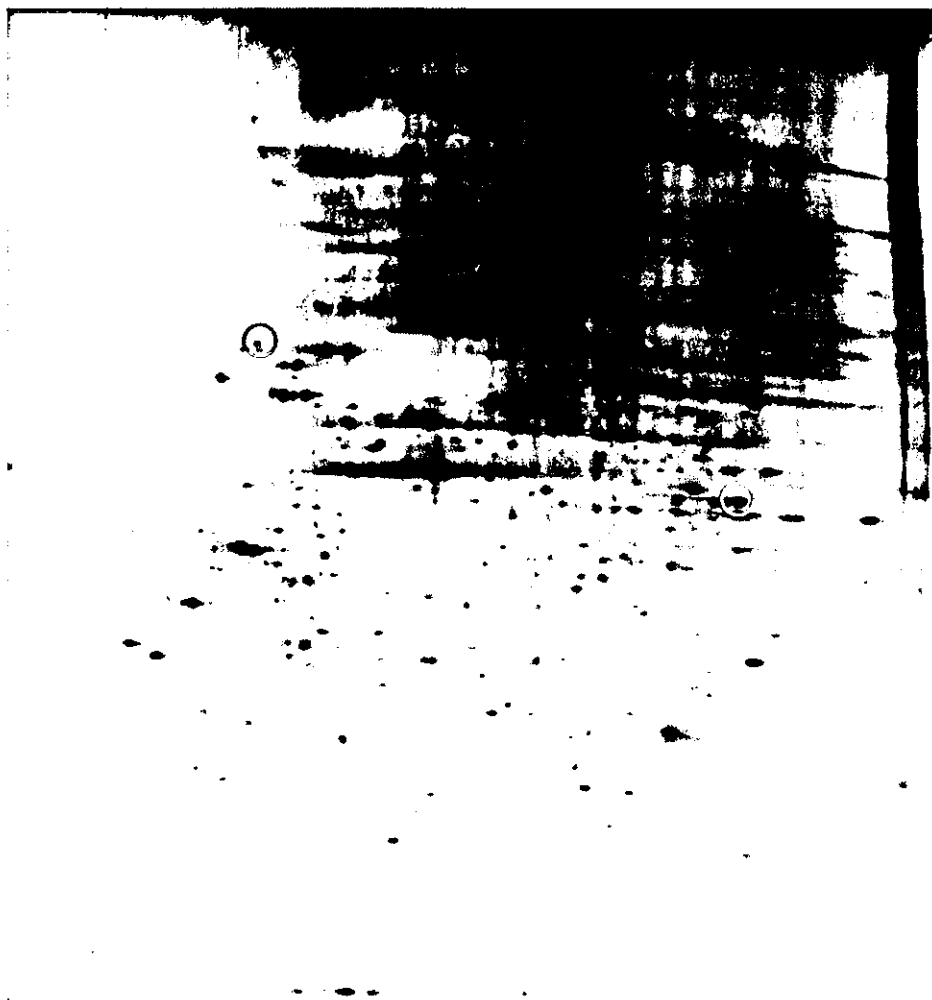


図-17
Rcho-1 細胞核抽出液の2次元電気泳動像(DES処理)。Rcho-1細胞をDESを含む培養液で培養し、その核抽出液を展開、銀染色した。赤○は対照群に比べて有意にコンテンツの上昇したスポットを示す。

ICI182780

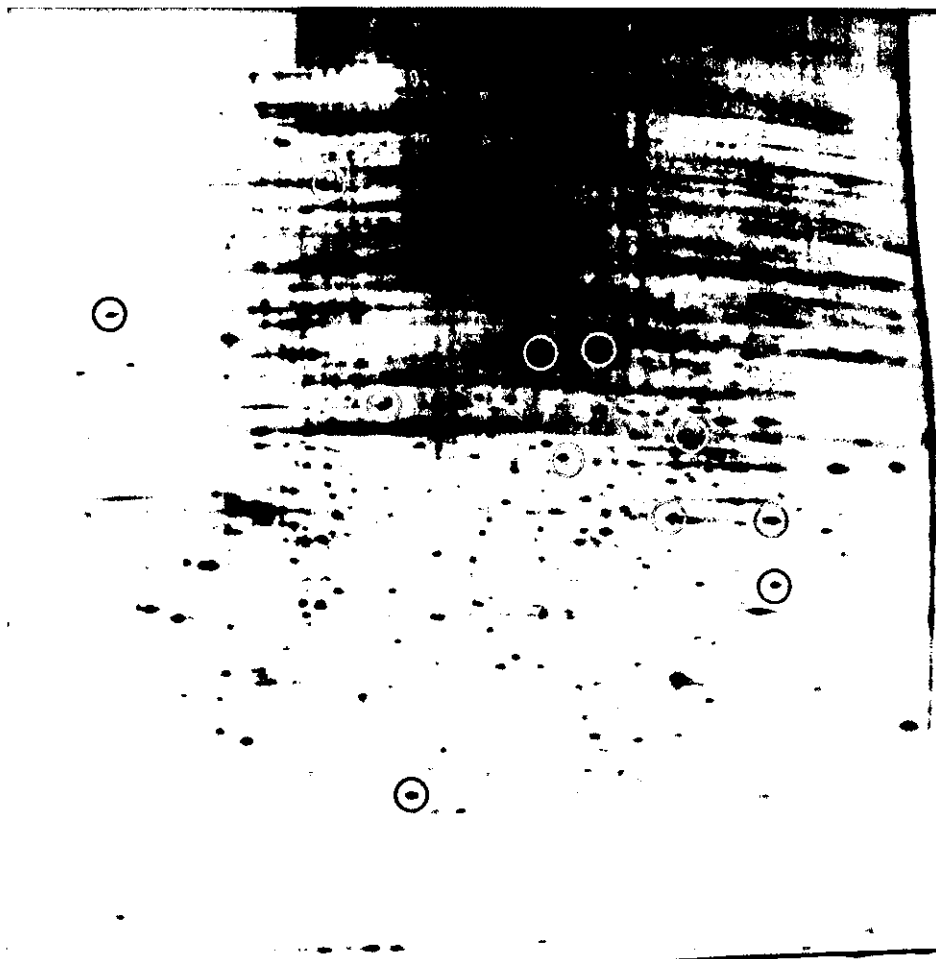


図-18
Rcho-1 細胞核抽出液の2次元電気泳動像(ICI処理)。Rcho-1細胞をICIを含む培養液で培養し、その核抽出液を展開、銀染色した。赤○は対照群に比べて有意にコンテンツの上昇したスポットを示す。

Carbaryl

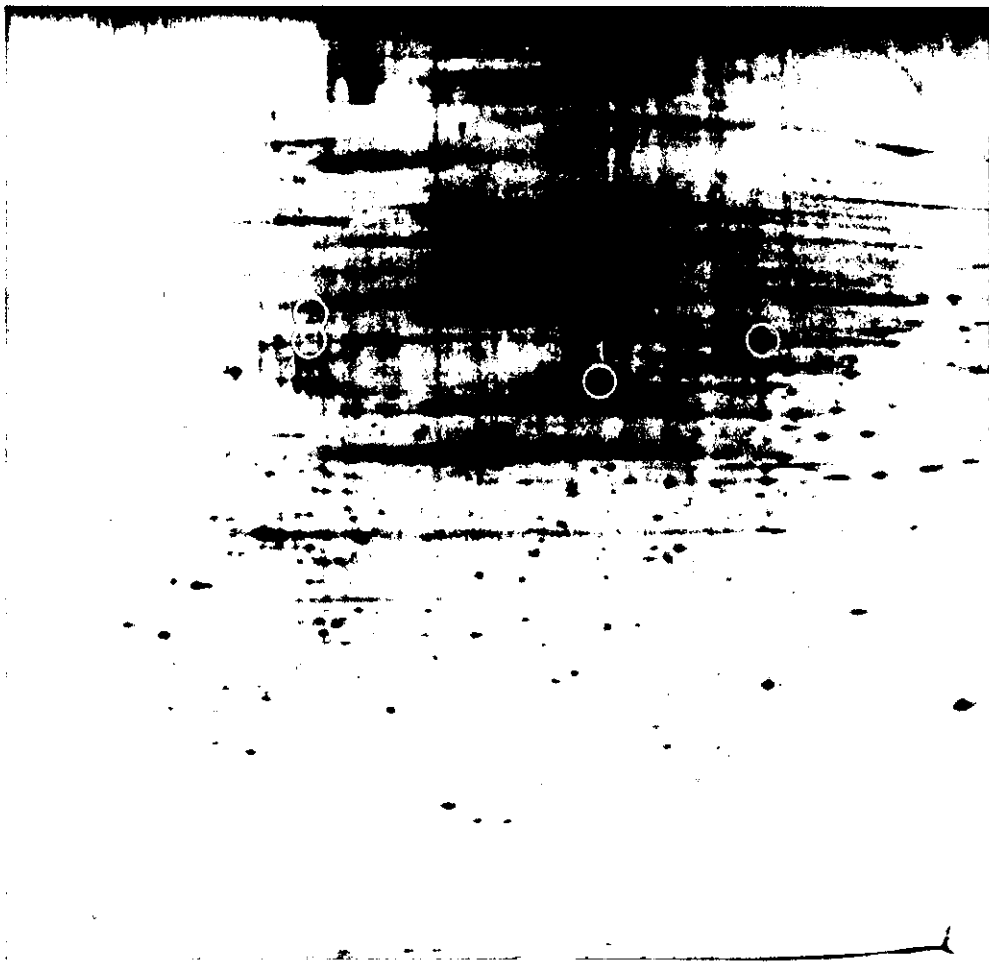
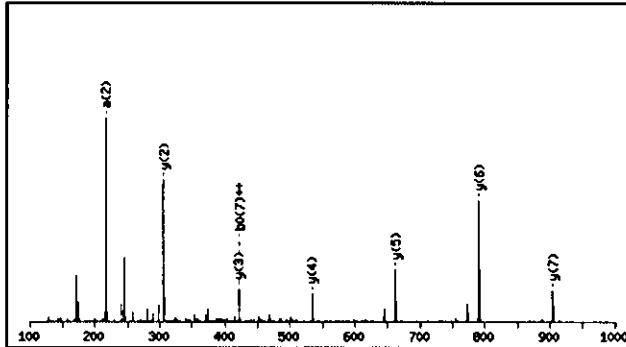


図-19

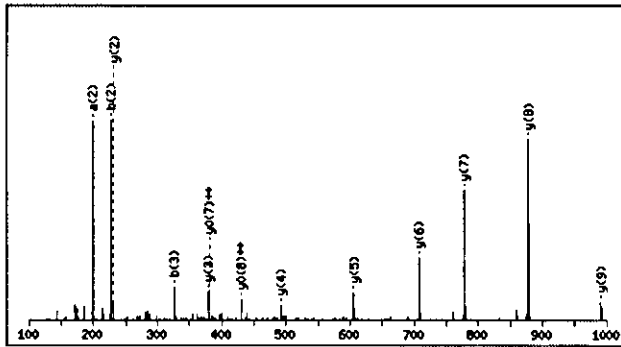
Rcho-1 細胞核抽出液の2次元電気泳動像(Carbaryl処理)。Rcho-1細胞をCarbarylを含む培養液で培養し、その核抽出液を展開、銀染色した。赤○は対照群に比べて有意にコンテンツの上昇したスポットを示す。プロテオミクス解析に供したスポットの番号を付加した。

A



#	a	a ⁺⁺	b	b ⁺⁺	b ⁺	b ⁺⁺⁺	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y ⁺	y ⁺⁺⁺	y ⁰	y ⁰⁺⁺	#
1	104.05	52.53	132.05	66.53					M							8
2	217.14	109.07	245.13	123.07					L	904.46	452.73	887.43	444.22	886.45	443.73	7
3	346.18	173.59	374.17	187.59			356.16	178.59	E	791.37	396.19	774.35	387.68	773.36	387.18	6
4	474.24	237.62	502.23	251.62	485.21	243.11	484.22	242.62	Q	662.33	331.67	645.30	323.16	644.32	322.66	5
5	587.32	294.17	615.32	308.16	598.29	299.65	597.31	299.16	L	534.27	267.64	517.24	259.13	516.26	258.63	4
6	702.35	351.68	730.34	365.68	713.32	357.16	712.33	356.67	D	421.19	211.10	404.16	202.58	403.18	202.09	3
7	833.39	417.20	861.39	431.20	844.36	422.68	843.37	422.19	M	306.16	153.58	289.13	145.07			2
8									R	175.12	88.06	158.09	79.55			1

B



#	a	a ⁺⁺	b	b ⁺⁺	b ⁺	b ⁺⁺⁺	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y ⁺	y ⁺⁺⁺	y ⁰	y ⁰⁺⁺	#
1	86.10	43.55	114.09	57.55					I							10
2	199.18	100.09	227.18	114.09					L	990.57	495.79	973.55	487.28	972.56	486.79	9
3	298.25	149.63	326.24	163.63					V	877.49	439.25	860.46	430.74	859.48	430.24	8
4	369.29	185.15	397.28	199.14					A	778.42	389.71	761.39	381.20	760.41	380.71	7
5	470.33	235.67	498.33	249.67			480.32	240.66	T	707.38	354.20	690.36	345.68	689.37	345.19	6
6	584.38	292.69	612.37	306.69	595.35	298.18	594.36	297.68	N	606.34	303.67	589.31	295.16			5
7	697.46	349.23	725.46	363.23	708.43	354.72	707.45	354.23	L	492.29	246.65	475.27	238.14			4
8	844.53	422.77	872.52	436.77	855.50	428.25	854.51	427.76	F	379.21	190.11	362.18	181.60			3
9	901.55	451.28	929.55	465.28	912.52	456.76	911.54	456.27	G	232.14	116.57	215.11	108.06			2
10									R	175.12	88.06	158.09	79.55			1

図-20

Rcho-1 細胞核抽出液(Carbaryl処理)に見られたスポット1のLC-MS/MS解析結果(ペプチドビュー)。Q-TOF解析によるペプチド断片2種のMS/MSスペクトルとイオンスコア(a, b, yシリーズ)、ならびに決定されたアミノ酸配列を示した。

Protein View

Match to: **gi|55562871**; Score: 307

Nuclear RNA helicase, DECD variant of DEAD box family [Rattus norvegicus]

Found in search of \\Qug\spro2.PRO\OutputESI\pl05030902.pkl

Nominal mass (M_r): 49078; Calculated pI value: 5.46

NCBI BLAST search of **gi|55562871** against nr

Unformatted sequence string for pasting into other applications

Links to retrieve other entries containing this sequence from NCBI Entrez:

gi|55926219 (no taxonomy information for this entry)

Variable modifications: Acetyl (N-term), Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: 16%

Matched peptides shown in Bold Red

```

1 MAEQDVENEL LDYDEDEEPO VPQESTPAPP KKDVKGSYVS IHSSGFRDFL
51 LKPELLRAIV DCGFEHPSEV QHECIPOAIL GMDVLCQAKS GMGKTAVFVL
101 ATLOQIEPIN GOVSVLVMCH TRELAFQISK EYERFSKYMP SVKVSVFFGG
151 LSIKKDEDVL KKNCPHVYVG TPGRILALVR SRSLNLRNVK HFVLDECDKM
201 LEQLDMRRDV QEIFRLPHE KQCMFSAITL SKEIRPVCRK FMQDPMEVFV
251 DDETKLTLHG LCOYYVKLKD SEKNRKLFDL LDVLEFNQVV IFVKSQRCM
301 ALAQLLVEQN FPAIAIHRGM AQEERLSRYQ QFKDFQRRIL VATNLFGRGM
351 DIERVNIVFN YDMPEDSDTY LHRVARAGRF GTKGLAVTFV SDENDAKILN
401 DVQDRFEVNV AELPEEIDIS TYIEQSR
  
```

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
48 - 57	622.35	1242.69	1242.73	-0.05	0	DFLKPELLR (Ions score 30)
123 - 130	468.25	934.48	934.51	-0.03	0	ELAFQISK (Ions score 34)
200 - 207	518.23	1034.45	1034.49	-0.04	0	MLEQLDMR (Ions score 54)
209 - 215	453.72	905.42	905.46	-0.04	0	DVQEIFR (Ions score 32)
256 - 267	488.25	1461.73	1461.80	-0.07	0	LTLHGLCQYYVK (Ions score 46)
339 - 348	552.31	1102.60	1102.65	-0.05	0	ILVATNLFGR (Ions score 67)
384 - 397	733.34	1464.67	1464.71	-0.04	0	GLAVTFVSCENDAK (Ions score 43)

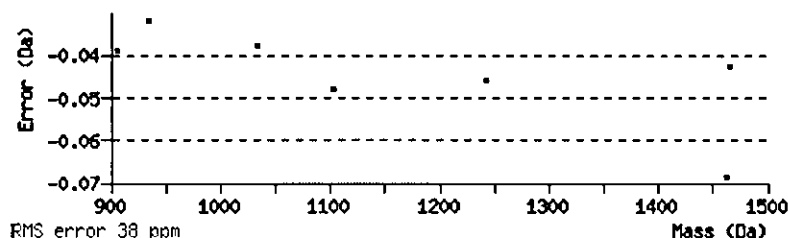


図-21

Rcho-1 細胞核抽出液(Carbaryl処理)に見られた誘導性スポット1のnanoLC-MS/MS解析のMascot検索結果(タンパクとしてNuclear RNA helicaseファミリーがラットデータベースにより検索された。分子量、等電点ともに2次元電気泳動スポット情報と一致していた。得られたペプチド断片シーケンスが赤で示されている。

E. 参考文献

- Safe, S.H., Pallaroni, L., Yoon, K., Gaido, K., Ross, S., and McDonnell D. Problems for risk assessment of endocrine-active estrogenic compounds. *Environ Health Perspect* 110, 925-929, (2002)..
- Tremblay, G. B., Kunath, T., Bergeron, D., Lapointe, L., Champigny, C., Bader, J. A., Rossant, J., and Giguere, V. Diethylstilbestrol regulates trophoblast stem cell differentiation as a ligand of orphan nuclear receptor ERR beta. *Genes Dev* 15, 833-838, (2001).
- Coward, P., Lee, D., Hull, M.V., and Lehmann, J.M. 4-Hydroxytamoxifen binds to and deactivates the estrogen-related receptor gamma. *Proc Natl Acad Sci U S A* 98: 8880-8884, (2001).
- Faria, T. N., and Soares, M. J. Trophoblast cell differentiation: establishment, characterization, and modulation of a rat trophoblast cell line expressing members of the placental prolactin family. *Endocrinology* 129, 2895-2906, (1991).
- Hamlin, G.P., Lu, X.J., Roby, K.F., and Soares, M.J. Recapitulation of the pathway for trophoblast giant cell differentiation in vitro: stage-specific expression of members of the prolactin gene family. *Endocrinology* 134, 2390-2396, (1994).
- Tanaka, S., Kunath, T., Hadjantonakis, A. K., Nagy, A., and Rossant, J. Promotion of trophoblast stem cell proliferation by FGF4. *Science* 282, 2072-2075, (1998).
- Ishimura, R., Yoshida, K., Kimura, H., Dohmae, N., Takio, K., Ogawa, T., Tanaka, S., and Shiota, K. Stage-specific modification of G protein beta subunits in rat placenta. *Mol Cell Endocrinol* 174, 77-89, (2001).
- Yamamoto, T., Roby, K.F., Kwok, S.C., and Soares, M.J. Transcriptional activation of cytochrome P450 side chain cleavage enzyme expression during trophoblast cell differentiation. *J Biol Chem* 269, 6517-6523, (1994).
- Yamamoto, T., Chapman, B.M., Clemens, J.W., Richards, J.S., and Soares, M.J. Analysis of cytochrome P-450 side-chain cleavage gene promoter activation during trophoblast cell differentiation. *Mol Cell Endocrinol* 113, 183-194, (1995).
- Nestler, J.E. Regulation of the aromatase activity of human placental cytotrophoblasts by insulin, insulin-like growth factor-I, and -II. *J Steroid Biochem Mol Biol* 44, 449-457, (1993).
- Siiteri, P.K., and Thompson, E.A. Studies of human placental aromatase. *J Steroid Biochem* 6, 317-322, (1975).
- Warshaw, M.L., Johnson, D.C., Khan, I., Eckstein, B., and Gibori, G. Placental secretion of androgens in the rat. *Endocrinology* 119, 2642-2648, (1986).

- Durkee, T.J., McLean, M.P., Hales, D.B., Payne, A.H., Waterman, M.R., Khan, I., and Gibori, G. P450(17 alpha) and P450SCC gene expression and regulation in the rat placenta. *Endocrinology* 130, 1309-1317, (1992).
- Yan, J., Tanaka, S., Oda, M., Makino, T., Ohgane, J., and Shiota, K. Retinoic acid promotes differentiation of trophoblast stem cells to a giant cell fate. *Dev Biol* 235, 422-432, (2001).
- Klotz, D.M., Arnold, S.F., and McLachlan, J.A. Inhibition of 17 beta-estradiol and progesterone activity in human breast and endometrial cancer cells by carbamate insecticides. *Life Sci* 60, 1467-1475, (1997).
- Denison, M.S., Phelan, D., Winter, G.M., and Ziccardi, M.H. Carbaryl, a carbamate insecticide, is a ligand for the hepatic Ah (dioxin) receptor. *Toxicol Appl Pharmacol* 152, 406-414, (1998).
- Strausberg, R.L., Feingold, E.A., Grouse, L.H., Derge, J.G., Klausner, R.D., Collins, F.S., Wagner, L., Shenmen, C.M., Schuler, G.D., Altschul, S.F., Zeeberg, B., Buetow, K.H., Schaefer, C.F., Bhat, N.K., Hopkins, R.F., Jordan, H., Moore, T., Max, S.I., Wang, J., Hsieh, F., Diatchenko, L., Marusina, K., Farmer, A.A., Rubin, G.M., Hong, L., Stapleton, M., Soares, M.B., Bonaldo, M.F., Casavant, T.L., Scheetz, T.E., Brownstein, M.J., Usdin, T.B., Toshiyuki, S., Carninci, P., Prange, C., Raha, S.S., Loquellano, N.A., Peters, G.J., Abramson, R.D., Mullahy, S.J., Bosak, S.A., McEwan, P.J., McKernan, K.J., Malek, J.A., Gunaratne, P.H., Richards, S., Worley, K.C., Hale, S., Garcia, A.M., Gay, L.J., Hulyk, S.W., Villalon, D.K., Muzny, D.M., Sodergren, E.J., Lu, X., Gibbs, R.A., Fahey, J., Helton, E., Kettman, M., Madan, A., Rodrigues, S., Sanchez, A., Whiting, M., Young, A.C., Shevchenko, Y., Bouffard, G.G., Blakesley, R.W., Touchman, J.W., Green, E.D., Dickson, M.C., Rodriguez, A.C., Grimwood, J., Schmutz, J., Myers, R.M., Butterfield, Y.S., Krzywinski, M.I., Skalska, U., Smailus, D.E., Schnerch, A., Schein, J.E., Jones, S.J., and Marra, M.A.. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc Natl Acad Sci USA* 99, 16899-16903, (2002).
- Bleoo, S., Sun, X., Hendzel, M.J., Rowe, J.M., Packer, M., and Godbout, R. Association of human DEAD box protein DDX1 with a cleavage stimulation factor involved in 3'-end processing of pre-mRNA. *Mol Biol Cell* 12, 3046-3059, (2001).
- Chen, H.C., Lin, W.C., Tsay, Y.G., Lee, S.C., and Chang, C.J. An RNA helicase, DDX1, interacting with poly(A) RNA and heterogeneous nuclear ribonucleoprotein K. *J Biol Chem* 277, 40403-40409, (2002).

F. 健康危機情報

なし

G. 研究発表

誌上発表

- Ishimura R, Ohsako S, Kawakami T, Sakaue M, Aoki Y, Tohyama C. Altered protein profile and possible hypoxia in the placenta of 2,3,7,8-tetrachlorodibenzo-p-dioxin-exposed rats. *Toxicol Appl Pharmacol.* 2002;185(3):197-206.
- Ohsako S, Miyabara Y, Sakaue M, Ishimura R, Kakeyama M, Izumi H, Yonemoto J, Tohyama C. Developmental stage-specific effects of perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on reproductive organs of male rat offspring. *Toxicol Sci.* 2002;66(2):283-292.
- Ishimura R, Ohsako S, Miyabara Y, Sakaue M, Kawakami T, Aoki Y, Yonemoto J, Tohyama C. Increased glycogen content and glucose transporter 3 (GLUT3) mRNA level in the placenta of Holtzman rats after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Appl pharmacol.* 2002;178:161-171.
- Sakaue M, Ishimura R, Kurosawa S, Fukuzawa HN, Kurohmaru M, Hayashi Y, Tohyama C, Ohsako S. Administration of estradiol-3-benzoate down-regulates the expression of testicular steroidogenic enzyme genes for testosterone production in the adult rat. *J Vet Med Sci.* 2002;64(2):107-113
- Fukuzawa H.N., Ohsako S., Nagano R., Sakaue M., Baba T., Aoki Y., and Tohyama C. Effects of 3,3',4,4',5-pentachlorobiphenyl, a coplanar polychlorinated biphenyl congener, on cultured neonatal mouse testis. *Toxicol in Vitro* 17, 259-269, (2003).
- Ohsako S., Kubota K., Kurosawa S., Takeda K., Wu Q., Ishimura R., and Tohyama C. Alterations of gene expression in adult male rat testis and pituitary shortly after subacute

administration of the antiandrogen flutamide. *J Reprod Dev* 49, 275-290, (2003).

Kubota K., Ohsako S., Kurosawa S., Takeda K.,
Wu Q., Sakaue M., Kawakami T., Ishimura R.,
and Tohyama C. Effects of vinclozolin
administration on sperm production and
testosterone biosynthetic pathway in adult male
rat. *J Reprod Dev* 49, 403-412, (2003).

Fukuzawa H.N., Ohsako S., Sakaue M., Baba
T., and Tohyama C. Aryl-hydrocarbon receptor
mediated down-regulation of cytochrome P450
side chain cleavage enzyme expression in the
adult male mice testis. *Mol Cell Endocrinol* 221,
87-96, (2004).

H. 知的所有権の取得状況

なし

Classic DFS, 6 mm for 31-60 sheets 446
www.bindomatic.com