KURABO

Research use only

Human cancer cell primary culture system kit

Primaster®

Specialized kit enabling 3D culture for CD-DST

This *in vitro* test kit's substrate recovers cancer cells efficiently from tumor tissues and reagents needed to conduct a chemosensitivity testing; CD-DST.

Applications

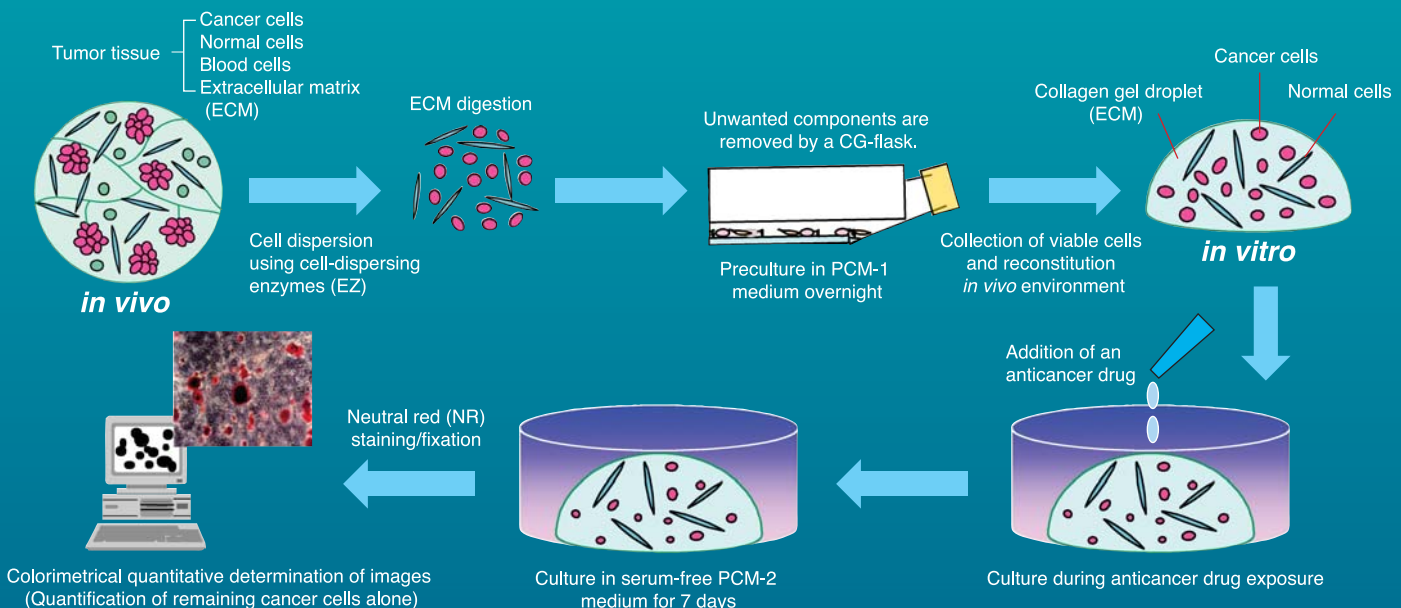
- ***In vitro* assay for predicting anticancer drug efficacy**
- **Screening for new candidate drugs during preclinical studies**
- **Chemosensitivity testing: Specialized assay kit for CD-DST**
  Primaster enables anticancer drug efficacy to be evaluated for a chemosensitivity from the primary tumor specimens.

Collagen gel droplet embedded culture drug sensitivity test

CD-DST "Individualized tumor response testing (ITRT)":

CD-DST represents 3D collagen-gel droplet-based culture mimic the biological environment and effectively and efficiently realizing primary human cancer cell culture.

CD-DST assay flow



Primaster®

• Evaluation at physiological drug concentrations

The exposure conditions in CD-DST

Drug	Concentration (μg/ml)	Exposure time (h)	Clinical dose	AUC (in vitro/human)[a]
MMC	0.03	24	20 mg/body	1.05
CDDP	0.2	24	100 mg/m²	1.67
DXR	0.02	24	60 mg/m²	0.98
VDS	0.01	24	2 mg/m²	0.8
VP-16	1.0	24	300 mg/m²×3	1.94
5-FU	1.0	24	600 mg/m²(bolus)	0.92
CBDCA	2.0	24	450 mg/m²	0.72
DOC	0.1	24	60 mg/m²	0.83
NVB	0.05	24	25 mg/m²	1.05
PAC	1.0	24	210 mg/m²	1.03
EPI	0.1	24	40 mg/m²	1.2

[a]1.0=same as clinical AUC.

A drug concentration at each in vitro assay

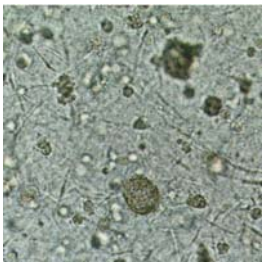
	CD-DST	MTT assay	HDRA	ATP assay
MMC	0.03	10	2.0	1
CDDP	0.2	20	20	2
DXR	0.02	4	15	0.4
5-FU	1.0	100	100	10
VP-16	1.0	-	300	10

(μg/ml)

• 3D cancer cell growth

Morphology of human primary breast cancer cell and fibroblast cell grown in each culture method

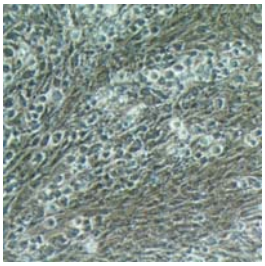
3D-culture



Collagen gel droplet

On day 7, cancer colonies are spherical in shape and fibroblasts are bipolar in shape.

Monolayer-culture

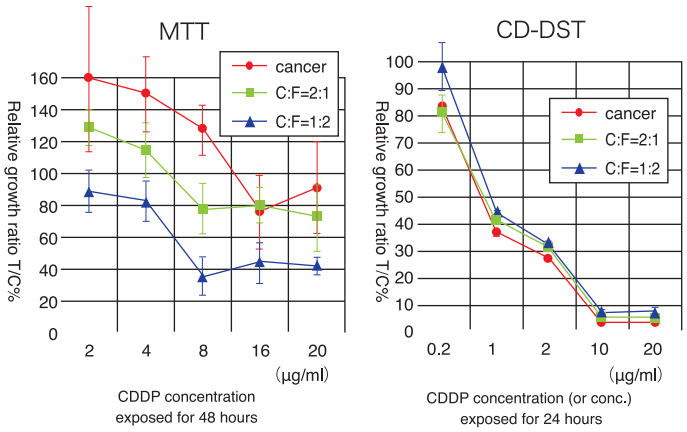


Monolayer

On day 4, fibroblast cells are grown outstandingly.
It is difficult to distinguish the cancer cells and fibroblast cells.

• Evaluation of anticancer effect on only cancer cells

Influence of contaminating fibroblast cells on anticancer effect



In the MTT assay, the antitumor effect is overestimated together with mixed fibroblast cell increases. On the other hand, CD-DST assay can evaluate the anticancer effect without an influence of contaminating fibroblast cells

*: Total cell number: 3 x 10^5cells

• High evaluation rate of surgical specimens

Evaluable rate of surgical specimens in the CD-DST

	NSCLC	Breast	Gastric	Colon	Others	Total
No. of cancers tested	659	163	92	107	70	1,091
No. of evaluable cases	543	132	78	89	64	906
Evaluable rate (%)	82	81	85	83	91	83

NSCLC:Non-small cell lung cancer
Growth rate of 0.8 or more was regarded as evaluable case.
Modified from 'Recent Results in Cancer Research 161, 48-61,2003.'

• Small number of cells required for CD-DST assay.

	No. of cell required per well	No. of proportional ratio as CD-DST as 1	Cell density	Cell dispersion solution required per well
CD-DST 1)	3×10^3 cells/well a)	1	1×10^5 cells/ml	30μl
MTT assay 2)	3×10^4 cells/well b)	10	3×10^5 cells/ml	100μl
ATP assay 3)	2×10^4 cells/well c)	6.7	2×10^5 cells/ml	100μl
EDRA 4)	3×10^4 cells/well d)	10	3×10^5 cells/ml	100μl

Plates used are a) 24 well-plate, b)c)d) 96 well-plate

1) Kobayashi H. et.al: Int. J. Oncol. 11, 449-455, 1997.
2) Carmichael J. et al: Cancer Res. 47, 936-942, 1987.
3) Kuzmits R. et al: J. Clin. Chem. Clin. Biochem. 24, 293-298, 1986.
4) Fruehauf JP. et al: Principles Practices Oncol Updates 7, 1-17, 1993;

Adapted cancer species:

Non-small cell lung cancer, breast cancer, gastric cancer, colorectal cancer, ovarian cancer, and cervical cancer.

Applicable anticancer drugs

CDDP, CBDCA, VNR, GEM, DOC, PAC, EPI, ADM, 5-FU, TS-1, MMC, VDS, CPT-11 (as SN-38), CPA (as 4-OH CPA)

KURABO INDUSTRIES LTD.
Biomedical Department Bio Reagents and Services Section
Osaka: 2-4-31 Kyutaro-machi, Chuo-ku, Osaka 541-8581, Japan
TEL: +81-6-6266-5010 FAX: +81-6-6266-5011
Tokyo: NOF Nihonbashi-honcho Bldg. 2F
2-7-1 Nihonbashi-honcho, Chuo-ku, Tokyo 103-0023, Japan
TEL: +81-3-3639-7077 FAX: +81-3-3639-6998
URL: http://www.kurabo.co.jp/bio/English/

Distributor

Ⓟ
'10.09.