Application: Controlling for non-heparan CBD-FGF-binding activity

Heparan Degrading Enzyme Assay Kit (Cat.# MK412)

The Heparan Degrading Enzyme Assay Kit employs a domain-oriented capture (DOC) method to detect the binding of undegraded heparan sulfate to the cell-binding domain of fibroblast growth factor (CBD-FGF) immobilized on the assay plate. If samples contain factors other than undegraded heparan that bind tightly to CBD-FGF, these factors may interfere with CBD-FGF binding to undegraded biotinylated heparan sulfate and hence reduce color development and assay read-out.

Therefore, to maintain accuracy it is necessary to confirm whether CBD-FGF binding activity not due to heparan is present in the assay system (for comparison, see list below of substances known to interact or not interact with FGF). The presence of this non-heparan CBD-FGF binding activity can be confirmed as follows:

1. In the absence of the biological sample (cell or tissue extract), incubate biotinylated heparan sulfate with and without the substance to be tested.
2. Transfer the two reactions into the wells of a microtiter plate containing immobilized CBD-FGF and determine the levels of color development.
3. If the two test reactions produce the same level of color development, no inhibiting activity is present. If the reaction with the test substance is lower than the heparan-only control, the substance binds to CBD-FGF.

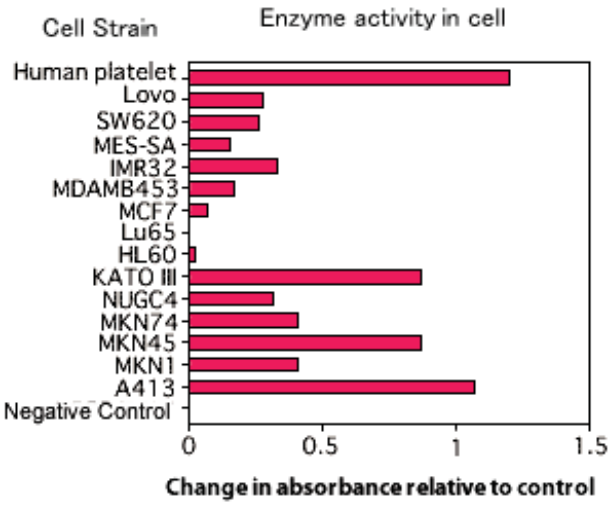
Alternatively, by utilizing this ability to detect inhibition of heparan binding, the kit can be used to screen for and study substances that have FGF-binding affinity. The following is a list of substances that Takara has confirmed to bind or not bind with FGF using this kit.

Interaction with FGF

Substances That Interact	That Do Not Interact
Dextran sulfate	Chondroitin sulfate A
Chondroitin sulfate B	Chondroitin sulfate C
Carrageenan Iota, κ, λ	Chondroitin sulfate D
	Keratan sulfate
	Pullulan
	Galactan
	Suramin

Assay Example

The levels of heparan sulfate degrading enzyme activity in various cultured cell extracts were measured using the Heparan Degrading Enzyme Assay Kit. Each cell sample (5×10^6 cells) was processed with 1 ml of Extraction Buffer and then diluted with reaction buffer prior to being assayed. The level of enzyme activity correlates with the extent to which the absorbance level of the sample is decreased relative to the negative control.



LoVo:	human colon adenocarcinoma
SW620:	human colon adenocarcinoma
MES-SA:	human uterus sarcoma
IMR32:	human neuroblastoma
MDAMB453:	human breast cancer
MCF7:	human breast cancer
Lu65:	human lung carcinoma
HL60:	human promyelocytic leukemia
KATOIII:	human gastric carcinoma
NUGC4:	human gastric carcinoma
MKN74:	human gastric carcinoma
MKN45:	human gastric carcinoma
MKN1:	human gastric carcinoma
A431:	human epidermoid
B16BL6:	mouse melanoma
10% FCS/RPMI:	negative control