

Rapid analysis of the extracellular matrix protein decorin using the Penta-His™ Alexa Fluor® 488 Conjugate

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Decorin is found in connective tissue where, among other roles, it helps to regulate cell growth by interacting with growth factors and collagen. After cloning of full-length human decorin cDNA into the pQE-TriSystem vector, human fibrosarcoma cells (HT-1080) were transfected, and the expression of decorin was analyzed by direct immunofluorescence using the Penta-His™ Alexa Fluor® 488 Conjugate.

Extracellular matrix (ECM) proteins play an essential role in regulation of differentiation, adhesion, and migration of cells and influence complex processes such as development, and tissue remodeling. Decorin is a member of the small leucine-rich family of proteoglycans, which are prominent constituents of both the ECM and the cell surface. Decorin is post-translationally modified by polymerization of a single glycosaminoglycan (GAG) chain onto a site near the N-terminus of the core protein and by N-glycosylation at three potential sites (1). It may modify vascular smooth muscle cell function by altering the response to growth factors, such as TGF- β 1, and the accumulation of ECM proteins during vascular injury (2–4). Decorin has been shown to influence the proliferative capacity of cells (5).

The goal of this study was to produce a biologically active decorin protein, consisting of both the core protein and the GAG chain. To achieve this, the protein's cDNA was cloned into the pQE-TriSystem vector and overexpressed as a His-tagged fusion protein in a mammalian expression system (human fibrosarcoma cells). Expression of the protein was analyzed in situ by direct immunofluorescence using the Penta-His Alexa Fluor 488 Conjugate.

Materials and methods

Total RNA was isolated from human fibroblasts and the full-length decorin cDNA, containing the endogenous signal sequence,

was amplified by RT-PCR using decorin-sequence-specific primers containing BamHI and EcoRI restriction sites. PCR products were ligated into a TA-ligation vector and subjected to BamHI and EcoRI digestion. Decorin cDNA was inserted into the BamHI – EcoRI site of pQE-TriSystem, and the vector was transiently transfected into human fibrosarcoma cells (HT-1080).

The Penta-His Alexa Fluor 488 Conjugate was used to analyze transfection efficiency of HT-1080 cells. HT-1080 transfectants were fixed 24 hours after transfection (2% paraformaldehyde in PBS, 15 minutes at room temperature) and incubated with a 1:200 dilution of Penta-His Alexa Fluor 488 Conjugate for 1 hour at 4°C. His-tagged decorin was visualized by fluorescence microscopy.

Results and discussion

Human fibrosarcoma cells (HT-1080) were transfected with pQE-TriSystem vector containing the full-length cDNA of human decorin. Expressed protein could be detected using Penta-His Alexa Fluor 488 Conjugate as early as 24 hours post-transfection. Figures 1 and 2 show the intracellular localization of His-tagged decorin. Decorin is distributed in the nuclear envelope, endoplasmic reticulum, or confined to cisternae in the Golgi body. In Figure 2, nuclei were counterstained with blue-fluorescent DAPI. Some nuclear staining was observed in nontransfected cells (data not shown). ►

References

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Direct Immunofluorescent Detection of His-Tagged Proteins

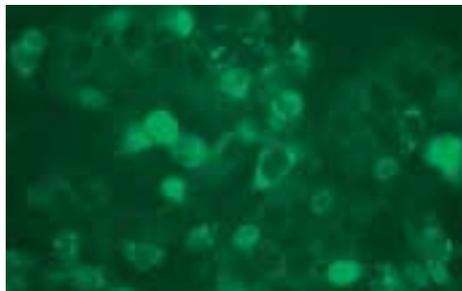


Figure 1 Fluorescence micrograph (400x) of human fibrosarcoma cells transfected with a pQE-TriSystem construct encoding His-tagged decorin. Cells were fixed with 2% paraformaldehyde 24 hours post-transfection and stained using Penta-His Alexa Fluor 488 Conjugate.

Sensitive and Specific Protein Localization

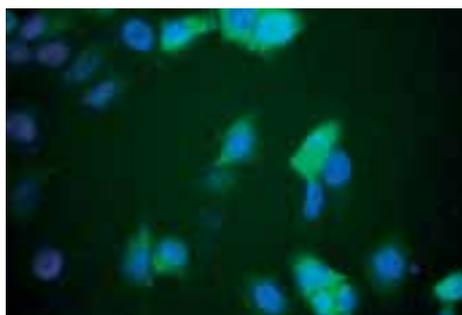


Figure 2 Fixed cells were stained using Penta-His Alexa Fluor 488 Conjugate and nuclei were counterstained with DAPI (400x).

Conclusions

- ◆ Expression in mammalian systems allows post-translational modification of over-expressed proteins. Post-translational modifications (e.g., signal sequence processing), may be essential for correct protein compartmentalization or function. The pQE-TriSystem vector contains 3 promoters allowing expression of His-tagged proteins in *E. coli*, insect, and mammalian cells using a single construct.
- ◆ The Penta-His antibody conjugated to green-fluorescent Alexa Fluor 488 is a sensitive and specific tool for localizing His-tagged proteins expressed in mammalian cells by direct immunofluorescence. Transfection efficiency, which is known to depend on several parameters such as cell type, size of transfected DNA, and time of transfection, can be easily checked. Such analyses can also be used to aid determination of protein expression levels before performing western blotting. ■

Ordering Information

Product	Contents	Cat. No.
For efficient expression of His-tagged proteins in <i>E. coli</i>, insect, or mammalian cells		
pQE-TriSystem Vector	25 µg pQE-TriSystem Vector DNA	33903
For direct detection of 6xHis-tagged proteins in immunofluorescence procedures		
Penta-His Alexa Fluor 488 Conjugate	125 µl Penta-His Alexa Fluor 488 Conjugate, 200 µg/ml	35310
Penta-His Alexa Fluor 532 Conjugate	125 µl Penta-His Alexa Fluor 532 Conjugate, 200 µg/ml	35330
Penta-His Alexa Fluor 555 Conjugate	125 µl Penta-His Alexa Fluor 555 Conjugate, 200 µg/ml	35350
Penta-His Alexa Fluor 647 Conjugate	125 µl Penta-His Alexa Fluor 647 Conjugate, 200 µg/ml	35370
For indirect detection of 6xHis-tagged proteins in immunofluorescence procedures		
Penta-His Biotin Conjugate	125 µl Penta-His Biotin Conjugate, 200 µg/ml	34440
Streptavidin-R-PE	250 µl Streptavidin-R-phycoerythrin Conjugate, 1 mg/ml	922721