Anti-Human CD90 (Thy-1) / Fibroblast Marker Mouse Monoclonal Antibody - unconjugated

Product Information

Catalog-No.: **DIA 100 (200 µg)**
DIA 100M (20 µg Sample)
Concentration: 0,2 mg/ml
Clone: AS02
Isotype: Mouse IgG_1, kappa
Specificity: Human CD90 (Thy-1)
(nerve cells, few $CD34^+$ blood stem cells, fibroblasts)
Immunogen: Human skin fibroblasts
Physical State: lyophilized powder
Reconstitution: After opening, restore to 1,1 ml (Sample 110µl) with sterile distilled water. The product has been overfilled to ensure recovery of total quantity.
Presentation: Mouse IgG1 in PBS with 2% BSA and 0,05% NaN_3, pH 7,4.
The antibody was purified from culture supernatant by mouse IgG-specific affinity chromatography.

Species Reactivity
Human
No reaction with Rat (cultured fibroblasts, FC), Mouse (fibrosarcom cell line L929, FC), Rabbit (skin, IH), Pig (skin, IH), Monkey (skin, IH)
Others not tested

Applications	Dilution
Flow Cytometry	1:50 – 1:200
Immunohistochemistry (Frozen Sections only)	1:50 – 1:200
Immunofluorescence	1:50 – 1:200
Immunoprecipitation / Cell Separaration	1:50 – 1:200
Immunoblot, nonreduced	5 – 10 ng/ml

The indicated dilutions are general recommendations. For special applications optimal working dilutions should be determined individually.

Reactivity

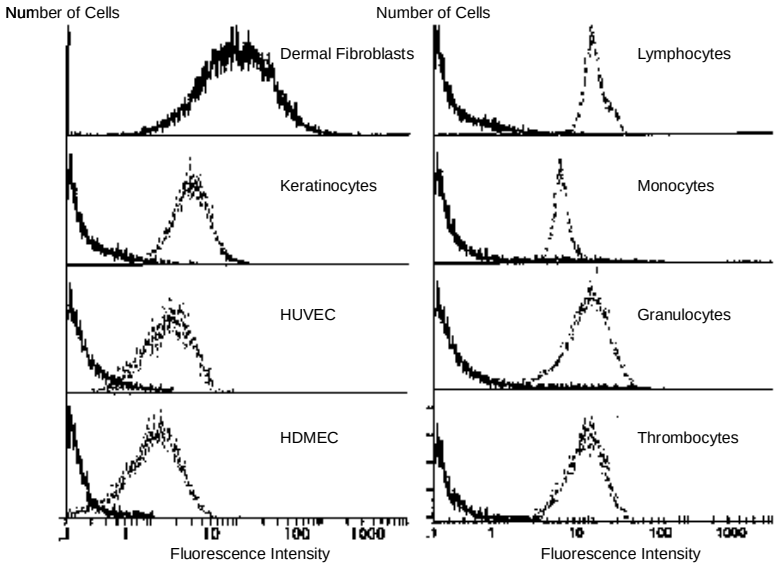
The monoclonal antibody clone AS02 reacts specifically with human CD90 (Thy-1), a GPI-anchored glycoprotein of the immunoglobulin superfamily with a molecular weight of 25 – 35 kDa (3). CD90 (Thy-1) in human is primarily expressed by nerve cells, additionally in a sub-population (20%) of $CD34^+$ blood stem cells and in various fibroblasts (1-8). In contrast to mouse and rat it is not expressed by thymocytes and peripheral blood T cells. AS02 recognizes fibroblasts of different origin but does not react with human blood cells, keratinocytes, resting micro-/macrovascular endothelial cells and components of the extracellular matrix like fibronectin, collagen type I, III, IV and laminin. Thus, AS02 is especially suitable for the specific detection and cell separation of human fibroblasts (2). Activated endothelial cells, after stimulation in vitro or in tissue sections of inflamed tissue in vivo, bind AS02 with different intensities, implying that CD90 (Thy-1) can be considered to be an activation marker of human endothelial cells. Recent papers demonstrate the binding of AS02 with activated microvascular endothelial cells (4,5), with a subpopulation of lung fibroblasts (6), specialized lymph node fibroblasts, but not lymphocytes (7) and normal or inflamed synovial fibroblasts in human (8). In immunoblot analysis AS02 detects nonreduced CD90 at 30 kDa (1,3).

Reactivity of AS02 in immunohistology

Skin	Fibroblasts	+	**Lymph Nodes**	Fibroblasts	+
	Keratinocytes	-		Lymphocytes	-
	Endothelial Cells	-	**Thyroid Gland/ Liver/ Kidney/ Gall Bladder**	Fibroblasts	+
	Smooth Muscle Cells	-		Epithelial Cells	-
	Glandular Cells	-		Thyrocytes	-
	Macrophages	-		Liver Parenchyma	-
	Langerhans Cells	-		Kidney Parenchyma	-
Cartilage	Chondrocytes	-		Tubulus Epithelial Cells	(+)
			Placenta	Fibroblasts (Mesenchyma)	+
Muscles	Smooth Muscle Cells	-		Endothelial Cells	-
	Heart Muscle Cells	-		Epithelial Cells	-
	Sceletal Muscle Cells	-	**Brain**	Nerve Cells	+

Legend: + strong binding; (+) recognition of few cells; - no binding

Reactivity of AS02 in flow cytometry



Binding of the monoclonal antibody AS02 to Dermal Fibroblasts in flow cytometrie. AS02 does not stain Lymphocytes, Keratinocytes, Monocytes, Granulocytes, Thrombocytes, HUVEC and HDMC. AS02 (———) compared to a an appropriate cell marker (--------); HUVEC = (Macrovascular) Human Umbilical Vein Endothelial Cells, HDMEC = Human Dermal Microvascular Endothelial Cells.

Instructions for Use

Applications

- Flow Cytometry
- Immunohistochemistry (Frozen Sections only)
- Immunofluorescence
- Immunoprecipitation / Cell Separation
- Immunoblot, nonreduced samples

Immunoblot

1. Pure or partially purified Thy-1* extracted from fibroblasts in Laemmli sample buffer without reducing agent, 3 min 96°C (*Campbell et al. Biochem J. 195(1):15-30 (1981); Etges et al. EMBO J. 5(3):597-601 (1986))
2. Cool sample on ice prior to application to a 0,75 x 6 x 8 cm 4 - 16% polyacrylamide gradient gel containing SDS
3. Transfer protein on PVDF membrane
4. Block with TBS/0,2% Tween20 + 2% BSA (TBST), 30 min RT
5. Incubate with 5 – 10 ng/ml AS02, 1 h RT
6. Wash with TBST, 3 x 5 min
7. Incubate with anti-mouse IgG (H+L) alkaline phosphatase (1:10,000), 1 h RT
8. Wash with TBST, 3 x 5 min
9. Add substrate BCIP/NBT

Separation of fibroblasts with magnetic beads

Contamination with fibroblasts is common in primary cultures of various cell types. Fast-growing fibroblasts can rapidly overgrow the desired cells (e.g. endothelial cells, keratinocytes, adrenal cortex cells, thyrocytes) in vitro, making it difficult to draw any meaningful conclusions from the resulting mixed cultures. Thus, a rapid and efficient means to eliminate fibroblasts is essential for reproducible results with primary cell cultures. AS02 coupled to magnetic beads allows an efficient means to remove fibroblasts from mixed cultures. Clearly, the same method allows the isolation of small numbers of fibroblasts from such cultures for further analysis.

Material

- Magnetic bead coupled secondary antibody anti-mouse (e.g. Dynabeads M-450 goat anti-mouse IgG, or sheep anti-mouse IgG beads from Advanced Biotechnologies Cat.-No. XMAS-205)
- Wash buffer: PBS/0,1% BSA and PBS/1% FCS
- Magnet holder for separation (e.g. Magnetic Separator Cat.-No. XS10/XS20 from Advanced Biotechnologies)
- Trypsin or EDTA

Coupling the fibroblast antibody AS02 to magnetic beads

1. Wash anti-mouse IgG magnetic beads, 3 x in PBS/0,1% BSA
2. Incubate with primary antibody AS02, 2 hours at RT or over night at 4°C at constant stirring (concentration of AS02 according to the binding capacity of the anti-mouse IgG magnetic beads)
3. Wash the anti-mouse IgG/ AS02 bead complex, 3 x in PBS/0,1% BSA

Separation of fibroblasts

1. Rinse cultivated cells, 3 x in PBS
2. Harvest cells from the surface of the culture dish by scraping or trypsin/EDTA treatment
3. Incubate cells with anti-mouse IgG/ AS02-coupled magnetic beads, 1 hour at RT at gentle agitation. For depletion the optimal ratio cells : beads is 1 : 10.
4. Separation of fibroblasts bound to magnetic beads by means of a magnetic separator. a) Positive selection: Wash fibroblasts, 3 x in PBS/1% FCS, and transfer into sterile culture tube. b) Negative selection: Transfer the fibroblast-free cell suspension in a sterile culture dish (after appropriate washing steps).

Storage and Stability

The antibody is stable for 1 year when stored as reconstituted liquid at 2-8°C.

References

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6. Hagood JS, Miller PJ, Lasky JA, Tousson A, Guo B, Fuller GM, McIntosh JC. Differential expression of platelet-derived growth factor-alpha receptor by Thy-1(-) and Thy-1(+) lung fibroblasts. *Am J Physiol* 277(1 Pt 1):L218-24, 1999.
7. Steiniger B, Barth P, Hellinger A. The perifollicular and marginal zones of the human splenic white pulp : do fibroblasts guide lymphocyte immigration? *Am J Pathol* 159(2):501-12, 2001.
8. Seemayer CA, Kuchen S, Kuenzler P, Rihoskova V, Rethage J, Aicher WK, Michel BA, Gay RE, Kyburz D, Neidhart M, Gay S. Seemayer CA, Kuchen S, Kuenzler P, Rihoskova V, Rethage J, Aicher WK, Michel BA, Gay RE, Kyburz D, Neidhart M, Gay S. Cartilage destruction mediated by synovial fibroblasts does not depend on proliferation in rheumatoid arthritis. *Am J Pathol* 162(5):1549-57, 2003.

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