Anti-Human CD90 (Thy-1) / Fibroblast Marker Mouse Monoclonal Antibody – FITC-conjugated

Product Information

Catalog-No.: **DIA 120, (200µg)**
Tests: Minimum 100 Tests (10 µl/ Test)
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 with sterile distilled water. The product has been overfilled to ensure recovery of total quantity.
Presentation: FITC-conjugated Mouse IgG1 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. FITC was coupled using standard procedures and unbound FITC was eliminated by gel 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 – 1:500 100 µl of an appropriate dilution for a suspension of 5 x 10^5 cells
(Immunofluorescence)	For 1:50 – 1:100 100 µl of an appropriate dilution per tissue section (frozen only) or monolayers of fixed cells

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

Reactivity

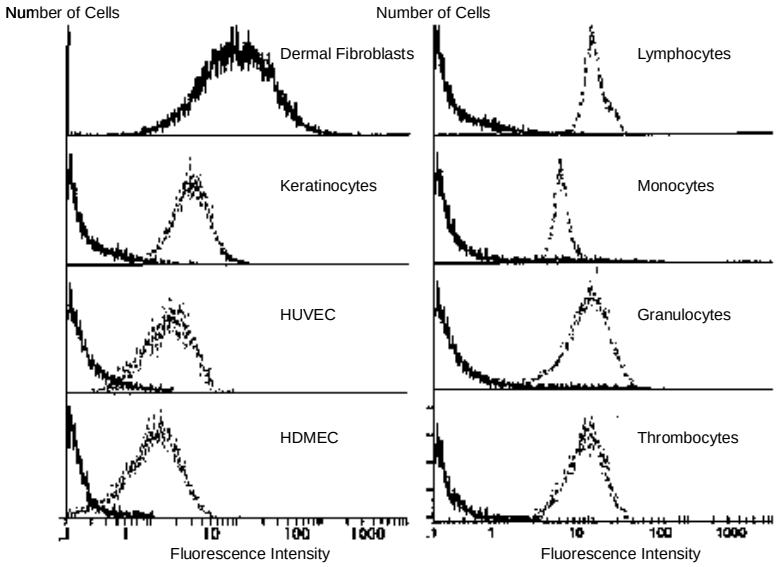
The monoclonal antibody clone AS02 reacts specifically with human CD90 (Thy-1), a GPI-anchored glycoprotein of the immunoglobulin superfamily with a molecular weight of 25 – 35 kDa (3). CD90 (Thy-1) in human is primarily expressed by nerve cells, additionally in a sub-population (20%) of $CD34^+$ blood 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

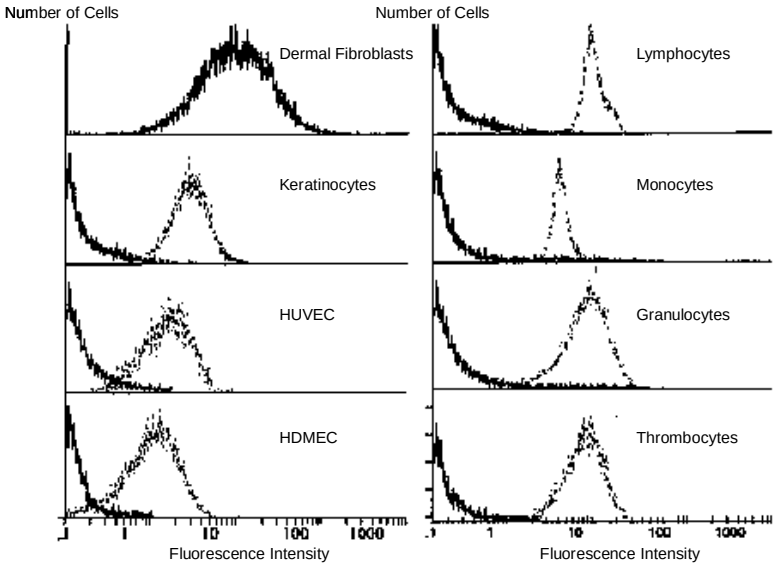
Skin	Fibroblasts	+	**Lymph Nodes**	Fibroblasts	+
	Keratinocytes	-		Lymphocytes	-
	Endothelial Cells	-	**Thyroid Gland/ Liver/ Kidney/ Gall Bladder**	Fibroblasts	+
	Smooth Muscle Cells	-		Epithelial Cells	-
	Glandular Cells	-		Thyrocytes	-
	Macrophages	-		Liver Parenchyma	-
	Langerhans Cells	-		Kidney 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

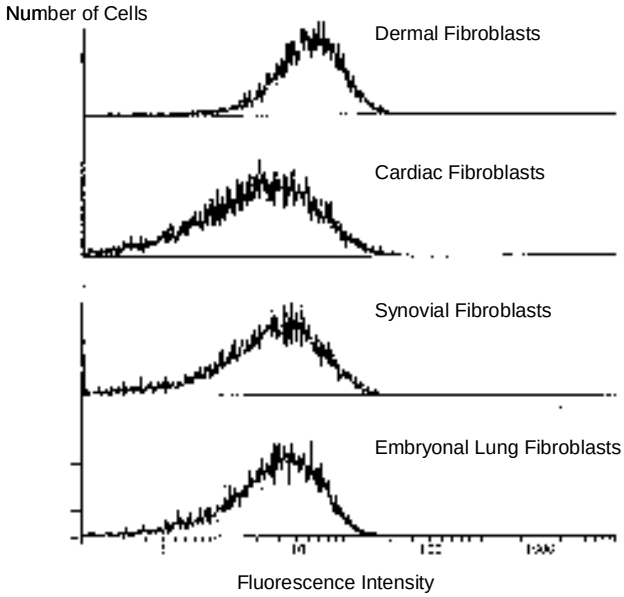
Reactivity of AS02 in flow cytometry



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.



Flow cytometric analysis of the binding capacity of mAb AS02 with different cell types.
Staining of different types of human cells with AS02 (———) compared to a corresponding negative control antibody (--------); HUVEC = (Macrovascular) Human Umbilical Vein Endothelial Cells, HDMEC = Human Dermal Microvascular Endothelial Cells.



Flow cytometric analysis of fibroblasts of different origin with mAb AS02.
Staining of different types of human fibroblasts with AS02 (———); only slight differences in the average fluorescence intenseness and therefore the binding affinity of monoclonal fibroblast antibody clone AS02 show the high affinity of this antibody to fibroblasts of different origin.

Instructions for Use

Applications

- Flow Cytometry
- Laser Scanning Cytometry
- Immunofluorescence
  (ethanol, methanol or acetone fixed cells and sections of frozen tissue only; formaldehyde-fixed paraffin embedded material is NOT stained by AS02)

1. Immunofluorescence

1. Cells: fix cells in ice-cold acetone for 5 minutes, allow to air dry. Frozen sections: warm slides at room temperature for 30 and fix in ice-cold acetone for 10 minutes, air dry for 5 (likewise ice-cold ethanol or methanol are suitable for fixation)
2. Rinse cells/sections in 3 changes of washing buffer; 5 minutes each
3. Optionally block nonspecific binding with 5% FCS for 20 at room temperature
4. Add 50 – 100 µl of FITC-conjugated anti-CD90 antibody AS02 (DIA 120), diluted 1:50 to 1:100 in PBS/1% BSA
5. Incubate at room temperature for 60 minutes
6. Rinse slides in 3 changes of washing buffer; 5 minutes each
7. Counterstain slides with DAPI
8. Coverslip with mounting medium (e.g. 80-90% glycerol/PBS + 2,5% DABCO) and seal with nail polish
9. Store slides in dark at 4 ºC

2. Flow Cytometry

1. Detach cultured fibroblasts by 0,025% trypsin/0,01% EDTA for 5 - 10 minutes at 37°C or by using 2,5 mM/L EDTA/PBS
2. Wash 2 x with cold PBS; centrifugation at 200 x g for 5 minutes each
3. Resuspend 2 - 5 x 10^5 cells in 100 µl of a 1:50 – 1:500 dilution of anti-CD90 antibody AS02 (DIA 120) in PBS/1% BSA
4. Incubate for 45 minutes at 4°C in dark
5. Wash cells 2 - 3 x with cold PBS; centrifugation at 200 x g for 5 minutes each
6. Resuspend cells and analyse directly by flow cytometry; for later analysis resuspend cells in 1% neutral buffered formalin, store in dark at 4°C and analyse within 24 hours

Indirect immunofluorescent staining of human dermal fibroblasts.

Indirect immunofluorescence with AS02 and anti-prolylhydroxylase antibody on cultured human dermal fibroblasts; staining with AS02 followed by goat anti-mouse IgG DTAF conjugate (green fluorescence) and the anti-prolylhydroxylase antibody with goat anti-mouse CY3 conjugate (yellow fluorescence). (magnification x 300)

AS02 homogenously stains the entire cell surface, including the numerous pseudopods. A clustering or intensification of staining at cell-cell contact sites was not demonstrated.

Confocal laser scanning microscopic analysis shows a sharp AS02 labeling of the cell membrane and a diffuse cytoplasmatic staining, suggesting that the antigen occurs predominantly at the cell surface, but also in the cytoplasm.

Storage and Stability

The antibody is stable for 9 months when stored as undiluted 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.
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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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