



ZytoLight®

Products for FISH analysis

Signal Interpretation Guide

ZytoLight[®] SPEC EWSR1/FLI1 TriCheck[™] Probe ZytoLight[®] SPEC FOXO1/PAX3 TriCheck[™] Probe ZytoLight[®] SPEC SS18/SSX1 TriCheck[™] Probe



FLI1-EWSR1 Fusion



PAX3-FOXO1 Fusion



SS18-SSX1 Fusion



Sarcoma Differential Diagnosis

Sarcomas are a heterogeneous group of relatively rare malignant tumors of mesenchymal origin which can develop at any site of the body. They account for nearly 21% of all pediatric solid malignant cancers and less than 1% of all adult solid malignant cancers¹. At least one-third of patients with soft tissue sarcoma die from tumor-related disease, most of them from lung metastases².

Differential diagnosis of soft tissue tumors is based on evaluation of histologic features and clinical correlation, supplemented by IHC stains for the detection of diverse biomarkers. In addition, molecular testing using, e.g., FISH has become an essential tool and is routinely utilized in the diagnosis of sarcomas³.

A large proportion of sarcomas is associated with specific genetic aberrations, such as translocations, mutations, and amplifications, which are helpful in the diagnosis^{3,4,5,6}. Specific recurrent translocations resulting in chimeric fusion genes have been identified in around one-third of sarcomas and are believed to be the primary event driving tumori-genesis³.

The TriCheck[™] Design



The only way to distinguish 3 different gene conditions!

Sarcoma TriChe<u>ck™ Evaluation Procedure</u>

- 1. Localize the invasive component of a sarcoma specimen on a corresponding H&E or IHC slide.
- 2. The area for counting should include clearly distinguishable and well distributed nuclei.
- 3. Count at least **100 non-overlapping** intact nuclei in two separate areas of a population of tumor cells in the invasive component of the tumor.
- 4. Rearranged if:
 - Distance between the separate green and the separate orange signal is > 1 signal diameter
 - > 15% of neoplastic cells rearranged*
- 5. Report if gene status is indeterminate due to e.g. artifacts, analytic testing failure, etc. or if gene status is discordant with other histopathologic findings and repeat test with another specimen.

based on: Bridge RS, et al. (2006) Mod Pathol 19(1): 1-8.

* The validation of FISH probes is required for each type of tissue that is intended to be tested in clinical practice since different tissue types exhibit different cell types with different nuclei diameters which may result in different cut off values. In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

Zyto*Light* [®] SPEC EWSR1/FLI1 TriCheck[™] Probe

Ewing sarcoma/peripheral neuroectodermal tumor (ES/PNET) is defined by the presence of EWSR1 gene translocations. 96% of the cases harbor rearrangements of the EWSR1 gene, the most common translocation being t(11;22) which results in the fusion of EWSR1 to FLI1 in over 90% of cases. Rarely, FUS-ERG or FUS-FEV fusions can be found in ES/PNET^{3, 4, 5, 6}.

For prognosis and appropriate treatment it is important to differentiate ES/PNET from classic neuroblastoma, Wilms tumor, and rhabdomyosarcoma. In combination with the histopathological diagnosis, detection of the EWSR1 rearrangements by FISH can be used to confirm the diagnosis of Ewing sarcoma/PNET⁸.



Zyto*Light* [®] SPEC FOXO1/PAX3 TriCheck[™] Probe

Rhabdomyosarcoma is the most common soft tissue sarcoma in children and young adults. Alveolar rhabdomyosarcoma (ARMS) can be distinguished from other sarcomas, including embryonal rhabdomyosarcoma (ERMS), because of two characteristic translocations, t(2;13) and t(1;13), which involve the FOXO1 (a.k.a. FKHR) gene and either PAX7 on chromosome 1p36 or PAX3 on chromosome 2q36.1. PAX7-FOXO1 is less common but is associated with a better prognosis^{2, 4, 5}.

The translocations and their fusion genes represent highly specific genetic markers useful in the diagnosis and prognosis of ARMS⁵.



ZytoLight [®] SPEC SS18/SSX1 TriCheck[™] Probe

All soft tissue tumors with a balanced SS18 translocation are considered synovial sarcomas. The translocation fuses SS18 (a.k.a. SYT) with either SSX1 (65%), SSX2 (35%), or more rarely with SSX4. Since malignant peripheral nerve sheath tumors, which share similar histologic appearance with synovial sarcomas, do not harbor SS18-translocations, FISH testing can support diagnosis of synovial sarcomas^{4, 5}.

Moreover, patients with SS18-SSX1 fusions were shown to have a higher risk of developing metastases compared to those with SS18-SSX2 fusions. Hence, detection of the SS18 fusion gene variant by FISH may also be of prognostic significance⁷.



- Burningham Z, et al. (2012) Clin Sarcoma Res 2: 14.
 WHO Classification of Tumours of Soft Tissue and Bone, 4th Edition (2013).
 Lauer S, Gardner JM (2013) Curr Probl Cancer 37: 45-61.
 Tanas MR, Goldblum JR (2009) Adv Anat Pathol 16: 383-91.
- Jain S, et al. (2010) Int J Clin Exp Pathol 3: 416-28.
 Chibon F, Coindre JM (2011) Pathologe 32: 32-9.
 Kawai A, et al. (1998) N Engl J Med 338: 153-60.
 Bridge RS, et al. (2006) Mod Pathol 19: 1-8.

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Products for FISH analysis

Zyto*Light* [®] Sarcoma TriCheck[™] Probes

Prod. No.	Product	Label	Tests* (Volume)	
Z-2183-50	Zyto <i>Light</i> SPEC EWSR1/FLI1 TriCheck™ Probe C€ [VD]	•/•/•	5 (50 µl)	
Z-2184-50	Zyto <i>Light</i> SPEC SS18/SSX1 TriCheck™ Probe C€ IVD	•/•/•	5 (50 µl)	
Z-2185-50	Zyto <i>Light</i> SPEC FOX01/PAX3 TriCheck™ Probe C€ IVD	●/●/●	5 (50 µl)	
Related Products				
Z-2028-5	Zyto <i>Light</i> FISH-Tissue Implementation Kit CE IVD		5	
	Incl. Heat Pretreatment Solution Citric, 150 ml; Pepsin Solution, 1 ml; Wash Buffer SSC, 210 ml; 25x Wash Buffer A, 50 ml; DAPI/DuraTect-Solution, 0.2 ml			
Z-2028-20	Zyto <i>Light</i> FISH-Tissue Implementation Kit CE IVD		20	
	Incl. Heat Pretreatment Solution Citric, 500 ml; Pepsin Solution, 4 ml; Wash Buffer SSC, 560 ml; 25x Wash Buffer A, 100 ml; DAPI/DuraTect-Solution, 0.8 ml			

* Using 10 µl probe solution per test. 🤇 ඟ only available in certain countries. All other countries research use only! Please contact your local dealer for more information

ZytoLight ® FISH-Tissue Implementation Kit

Zyto*Light* [®] FISH-Tissue Implementation Kit contains all neccessary reagents to perform user-friendly and successful FISH experiments.

- Heat Pretreatment Solution Citric
- Pepsin Solution
- Wash Buffer SSC
- 25x Wash Buffer A
- DAPI/DuraTect[™]-Solution



ZytoLight ® Fluorochromes

Two factors that mainly influence FISH analyses:

- $\cdot\,$ Fluorochromes of the FISH probes
- Appropriate filter sets

Fluorochrome Excitation Emission Equivalent to ZyBlue™ 418 nm 467 nm DEAC ZyGreen™ 503 nm 528 nm FITC Zy0range[™] 547 nm 572 nm Rhodamine

Recommended Filter Sets

All filter sets have a superior signal-tonoise ratio and need to be assembled in fluorescence filter holders specific for the respective microscope. Please contact info@zytovision.com for more information.

Prod. No	Product	Detected Fluorochrome
E-4030-1	DAPI Single Bandpass Filter Set v2	DAPI
E-4026-1	ZyBlue™ Single Bandpass Filter Set v2	•
E-4012-1	ZyGreen™ Single Bandpass Filter Set v2	•
E-4013-1	ZyOrange™ Single Bandpass Filter Set v2	•
E-4016-1	ZyGreen™/ZyOrange™ Dual Bandpass Filter Set v2	●/●

For more product information please contact info@zytovision.com or your local dealer

