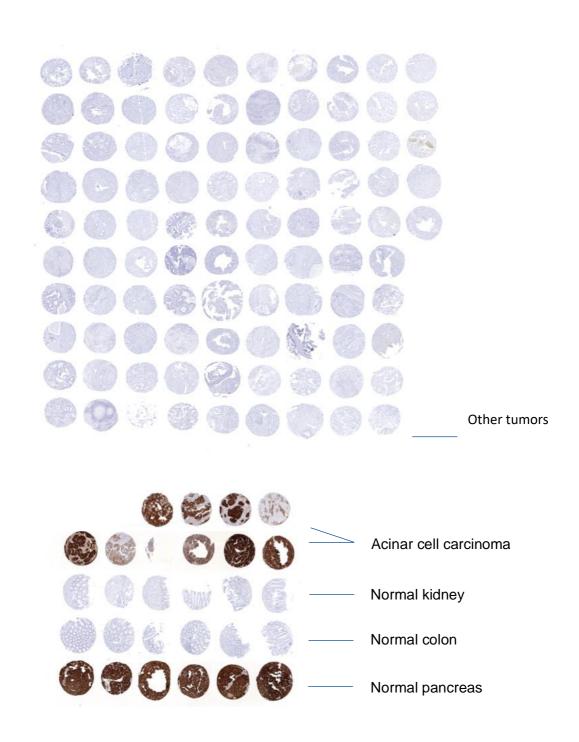


Department of Pathology

21.04.2022

LDT Manual – Caboxypeptidase A1 (CPA1): MSVA-601M



What is CPA1

Biology and staining pattern in normal tissues

Carboxypeptidase A1 (CPA1) is a zinc metalloprotease which is solely produced in acinar cells of the pancreas. CPA1 is released to the intestine and involved in zymogen inhibition and cleavage of aromatic amino acids from dietary proteins. RNA analyses of different kinds of normal tissues have confirmed exclusive expression of CPA1 in the pancreas (HPA).

Published data on tumors

A study by Uhlig et al. has described 100% sensitivity and 99.5% specificity of CPA1 expression analysis by using MSVA-601M for the identification of pancreatic acinar cell carcinomas in 12,274 tumors from 132 different tumor entities.¹ These data are very promising but given the still small number of analyzed pancreatic acinar cell carcinomas – a very rare tumor entity - it cannot be excluded that the sensitivity may be somewhat lower if more tumors are analyzed.

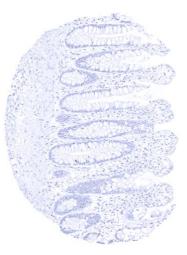
Characteristic Images



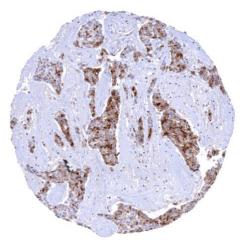
Strong CPA1 staining of normal pancreas (Note: Stroma is also staining due to contamination artifact).



Pancreatic acinar cell carcinoma showing strong positivity for CPA1 in all tumor cells. [©] UKE 2022



Complete lack of CPA1 staining in the normal colon mucosa.



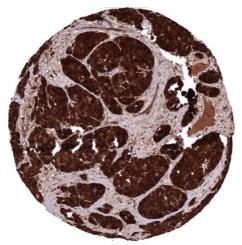
Pancreatic acinar cell carcinoma showing moderate staining for CPA1 in most tumor cells.

The complete results of Uhlig et al. are shown below: Data of Uhlig et al. from MSVA Homepage.^{1,2}

		0.0	10.0	20.0	30.0	% d 40.0	of tumors 50.0 60	0.0 70.	0 80.0	90.0	100.0
	Basal cell carcinoma (n=56)		10.0	20.0	30.0	40.0	50.0 60	.0 70.	0 80.0	90.0	100.0
	Squamous cell carcinoma of the skin (n=82)										
Tumors of the skin and head & neck	Malignant melanoma (n=43) Merkel cell carcinoma (n=44)										
nec	Squamous cell carcinoma of the larynx (n=95)										
of th id &	Squamous cell carcinoma of the pharynx (n=46)										
hea	Oral squamous cell carcinoma (floor of the mouth) (n=118) Acinic cell carcinoma of the salivary gland (n=128)										
and	Adenocarcinoma NOS of the salivary gland (n=69)										
	Adenoid cystic carcinoma of the salivary gland (n=99)										
	Epithelial-myoepithelial carcinoma of the salivary gland (n=51) Muccepidermoid carcinoma of the salivary gland (n=239)										
, p	Adenocarcinoma of the lung (n=160)										
I umors of the lung, pleura and thymus	Squamous cell carcinoma of the lung (n=65)										
ymu	Small cell carcinoma of the lung (n=16) Me sothelioma, epitheloid (n=32)										
1 <u>6</u> 1	Mesothelioma, other types (n=63)										
5	Thymoma (n=29)										
ри	Squamous cell carcinoma of the vagina (n=63) Squamous cell carcinoma of the vulva (n=44)										
ct a	Squamous cell carcinoma of the cervix (n=123)										
ll tra	Endometrioid endometrial carcinoma (n=223)										
inita	Endometrial serous carcinoma (n=72) Carcinosarcoma of the uterus (n=38)										
e ge st	Endometrioid carcinoma of the ovary (n=91)										
mal	Serous carcinoma of the ovary (n=462)										
e fe	Mucinous carcinoma of the ovary (n=71) Clear cell carcinoma of the ovary (n=40)										
ofth	Carcinosarcoma of the ovary (n=38)										
ors	Brenner tumor (n=9) Invasive breast carcinoma of no special type (n=1185)										
Tumors of the female genital tract and breast	Invasive breast carcinoma of no special type (n=1185) Lobular carcinoma of the breast (n=236)										
·	Mucinous carcinoma of the breast (n=44)										
E	Adenocarcinoma of the colon (n=722) Gastric adenocarcinoma, diffuse type (n=129)										
Tumors of the digestive system	Gastric adenocarcinoma, diffuse type (n=129) Gastric adenocarcinoma, intestinal type (n=134)										
esy	Adenocarcinoma of the esophagus (n=60)										
stiv	Squamous cell carcinoma of the esophagus (n=43) Squamous cell carcinoma of the anal canal (n=76)										
dige	Cholangiocellular carcinoma (n=107)										
the	Hepatocellular carcinoma (n=50)										
s of	Ductal adenocarcinoma of the pancreas (n=449) Pancreatic/Ampullary adenocarcinoma (n=75)										
ü	Acinar cell carcinoma of the pancreas (n=11)				_	_			_	_	_
Ъ	Mixed acinar endocrine carcinoma of the pancreas (n=1)								_		
	Gastrointestinal stromal tumor (GIST) (n=49) Urothelial carcinoma, pT2-4 G3 (n=612)										
Tumors of the urinary system	Small cell neuroendocrine carcinoma of the bladder (n=20)										
of t yste	Sarcomatoid urothelial carcinoma (n=24)										
ary s	Clear cell renal cell carcinoma (n=758) Papillary renal cell carcinoma (n=208)										
Tur Tur	Chromophobe renal cell carcinoma (n=118)										
	Oncocytoma (n=147)										
	Adenocarcinoma of the prostate (n=232) Small cell neuroendocrine carcinoma of the prostate (n=18)										
ali	Seminoma (n=444)										
ans	Embryonal carcinoma of the testis (n=39)										
orgi	Yolk sac tumor (n=32) Teratoma (n=44)										
male genital organs	Squamous cell carcinoma of the penis (n=66)										
	Adenoma of the thyroid gland (n=107) Papillary thyroid carcinoma (n=360)										
	Follicular thyroid carcinoma (n=360)										
Jans	Me dullary thyroid carcinoma (n=104)										
e orç	Anaplastic thyroid carcinoma (n=43) Adrenal cortical adenoma (n=44)										
Tumors of endocrine organs	Adrenal cortical carcinoma (n=44)										
	Phaeochromocytoma (n=50)										
	Appendix, neuroendocrine tumor (NET) (n=12) Colorectal, neuroendocrine tumor (NET) (n=10)										
OLS	Ileum, neuroendocrine tumor (NET) (n=10)										
Tum	Lung, neuroendocrine tumor (NET) (n=17)										
	Pancreas, neuroendocrine tumor (NET) (n=86) Colorectal, neuroendocrine carcinoma (NEC) (n=10)										
	Pancreas, neuroendocrine carcinoma (NEC) (n=10) Pancreas, neuroendocrine carcinoma (NEC) (n=14)										
σ	Hodgkin Lymphoma (n=76)										
c an ues	Small lymphocytic lymphoma, B-cell type (B-SLL/B-CLL) (n=30) Diffuse large B cell lymphoma (DLBCL) (n=94)										
tiss	Follicular lymphoma (DEBCE) (1=94)										
topo	T-cell Non Hodgkin lymphoma (n=16)										
haemotopoetic and lymphoid tissues	Mantie cell lymphoma (n=13) Marginal zone lymphoma (n=10)										
	Diffuse large B-cell lymphoma (DLBCL) in the testis (n=13)										
	Tenosynovial giant cell tumor (n=44)										
	Granular œll tumor (n=44) Leiomyoma (n=48)										
	Leiomyosarcoma (n=84)										
Tumors of soft tissue and bone	Liposarcoma (n=129)										
	Ma lignant peripheral nerve sheath tumor (MPNST) (n=11) Myofibrosarcoma (n=26)										
	Angiosarcoma (n=66)										
le a	Angiomyolipoma (n=91)										
lissu	Dermatofibrosarcoma protuberans (n=18) Ganglioneuroma (n=13)										
soft t	Kaposi sarcoma (n=6)										
ofs	Neurofibroma (n=96)										
lors	Sarcoma, not otherwise specified (NOS) (n=58) Paraganglioma (n=37)										
Tur	Ewing sarcoma (n=37)										
	Rhabdomyosarcoma (n=6)										
	Schwannoma (n=106) Synovial sarcoma (n=11)										
	Osteosarcoma (n=35)										

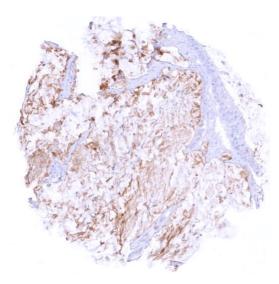
Potential pitfalls

-contamination artifacts: The CPA1 expression level in normal acinar cells is very high. Therefore, acinar-cell adjacent structures can show some staining ("pseudo-positivity") due to a diffusion of CPA1 protein from neighboring acinar cells. The spread of CPA1 protein may be facilitated by a mild tissue damage caused by early autolysis during ischemia time under surgery.

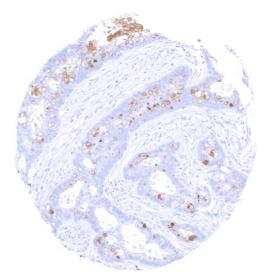


Pancreatic acinar cell carcinoma showing strong positivity for CPA1 in all tumor cells and also significant stroma staining (contamination artifact).

-mucus staining: Because CPA1 is released to the intestine some CPA1 staining can occasionally be found in mucus of the lower gastrointestinal tract. Very rarely, CPA1 staining can even be seen in goblet cells of colorectal carcinomas. Pancreatic origin of these mucins is supported by identical staining patters for Chymotrypsin (data not shown).



CPA1 staining of mucins in a colorectal adenocarcinoma.



CPA1 staining of mucins in goblet cells of a colorectal adenocarcinoma.

A) Justification of selection of MSVA-601M for CPA1 detection

MSVA-601 is the most extensively validated CPA1 antibody on the market and the validation data of this antibody are much broader than what is available for any other CPA1 antibody, irrespective of IVD label.

The antibody has been validated by Uhlig et al. according to the guidelines of the International working group of antibody validation (IWGAV) which requests orthogonal validation and/or a comparison with a different independent antibody for antibodies to be used on formalin fixed tissues.¹ The results of this validation are extensively documented and also explained on the homepage of the vendor under "evidence for antibody specificity".² No other vendor provides a comparable explanation of the validation process for a CPA1 antibody.

The antibody has been evaluated for cross-reactivity in >50 different normal tissues and the results have been described by Uhlig et al..¹ In addition, further images of these experiments are documented on the homepage of the vendor.³ No other CPA1 antibody has been evaluated that extensively for possible cross-reactivities and a comparable image documentation of the staining patterns in normal tissues is not available for other CPA1 antibodies.

The performance of the antibody in tumors has been comprehensively evaluated in a study by Uhlig et al. on 12,274 cancers from 132 different tumor entities.¹ No other CPA1 antibody has been evaluated for real life performance in a comparable number of tumors.

The vendor has described a protocol for DAKO autostainer Link48 that results in comparable staining results as obtained by the manual protocol of Uhlig et al..¹ The availability of dozens of images (tumors and normal tissues) obtained by the original protocol of the Uhlig study can serve as a reference for adjusting the own protocol. No other antibody comes with this level of documentation.

B) Relevant statements

A commercial CE-IVD marked anti CPA1 antibody with a comparable level of documentation of its performance characteristics is not available for the intended use.

The risk class of the antibody is class C according to rules 3f, 3h, and 3k of Appendix VIII EU regulation 2017/746.

Antibody MSVA-601M will be continuously supervised during usage so that corrective measures can be taken if necessary".

The antibody MSVA-601M meets the basic safety and performance requirements in accordance with Annex 1 of the IVDR.

This declaration is publicly available at:

C) Intended use of the antibody MSVA-601M

The antibody ABC is used for detection of CPA1 protein in formalin-fixed human tissue samples.

D) Protocol

Autostainer: Agilent / Dako – Autostainer Link 48

Procedure: Pretreatment in PT-Link for 30 minutes at 95°C (pH high); FLEX peroxidase blocking for 5 minutes (room temperature), MSVA-601M 1:150 for 20 minutes (room temperature), FLEX+ mouse/rabbit (LINKER) for 15 minutes (room temperature), horseradish peroxidase (HRP) for 20 minutes (room temperature), FLEX DAB+Sub-Chromo for 10 minutes (room temperature), FLEX hematoxylin for 5 minutes (room temperature).

E) Positive and negative control tissues

Positive tissue control: Pancreas: A strong staining of acinar cells should be seen. Adjacent structures can also be stained due to contamination artifacts.

Negative tissue control: Colon: CPA1 immunostaining must be absent in all cell types.

F) Assessment of precision

Use one block of normal pancreas and one block of normal colon

-day 1: stain 3 sections each of both blocks for CPA1 by MSVA-601M by using the predefined staining protocol (analysis of intra-run consistency)

-day 2: stain 3 sections each of both blocks for CPA1 by MSVA-601M by using the predefined staining protocol (analysis of inter-run consistency)

-day 3: stain 3 sections each of both blocks for CPA1 by MSVA-601M by using the predefined staining protocol (analysis of inter-run consistency)

All results are documented on the form (CPA1-staining: Precision assessment; page 8)

Expected result:

-Strong CPA1 positivity in all 9 sections from normal pancreas,

-Absence of CPA1 staining in all 9 sections from normal colon.

G) Assessment of sensitivity and specificity

Analyze 10 samples of normal pancreas (expected to be positive) and 10 samples of other normal tissues (colon, kidney, placenta, brain, skin, smooth muscle, liver, gallbladder, adrenal gland) which are all expected to be negative.

All results are documented on the form (CPA1-staining: Specificity/sensitivity assessment) which describes the necessary calculations for determining the specificity and sensitivity (page 9).

Expected result:

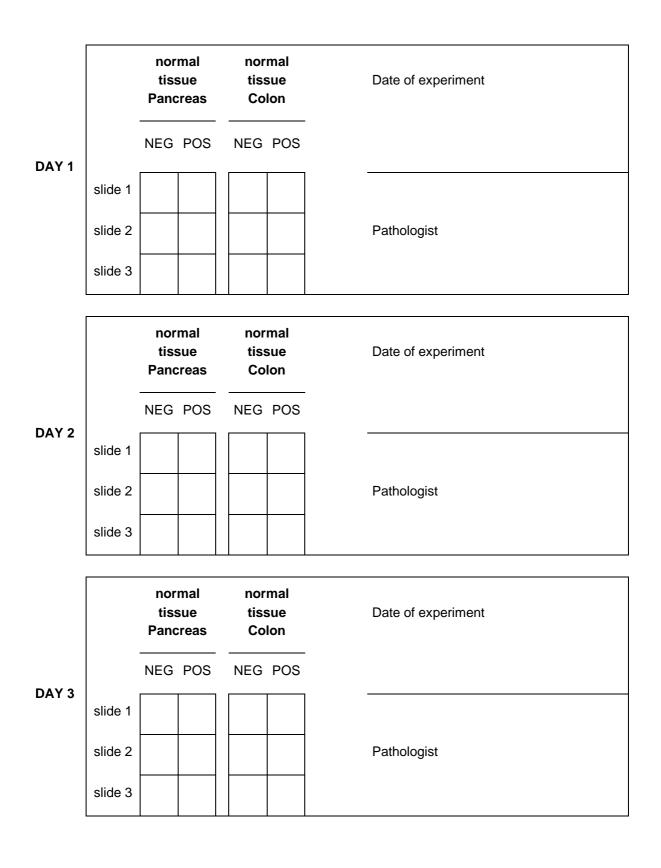
Sensitivity = 100%

Specificity = 100%

References:

- Uhlig R, Contreras H, Weidemann S, et al. Carboxypeptidase A1 (CPA1) Immunohistochemistry Is Highly Sensitive and Specific for Acinar Cell Carcinoma (ACC) of the Pancreas. Am J Surg Pathol. 2022;46(1):97-104. doi:10.1097/PAS.00000000001817
- 2. https://ms-validatedantibodies.com/product/cpa1-msva-601m/
- 3. https://ms-validatedantibodies.com/product-gallery/normal-tissue-gallery-cpa1/





Expected positive	IHC result		Expected negative	IHC resul	
tissue type	NEG	POS	tissue type	NEG	PC
Tissue Pancreas #1			Assumed neg tissue Colon		
Tissue Pancreas #2			Assumed neg tissue Kidney		
issue Pancreas #3			Assumed neg tissue Placenta		
Tissue Pancreas #4			Assumed neg tissue Brain		
Fissue Pancreas #5			Assumed neg tissue Skin		
Tissue Pancreas #6			Assumed neg tissue Smooth muscle		
Fissue Pancreas #7			Assumed neg tissue Liver		
īissue Pancreas #8			Assumed neg tissue Gallbladder		
issue Pancreas #9			Assumed neg tissue Adrenal gland		
⊺issue Pancreas #10			Assumed neg tissue Tonsil		
# of false negative			Sensitivity*		
f of true negative			Specificity**		
# of false positive					
Date of experiment					
Pathologist					

Form: CPA1 staining: Specificity/sensitivity assessment

* Sensitivty = number of true positive / (number of true positive + number of false negative)

**Specificity = number of true negative / (number of true negative + number of false positive)