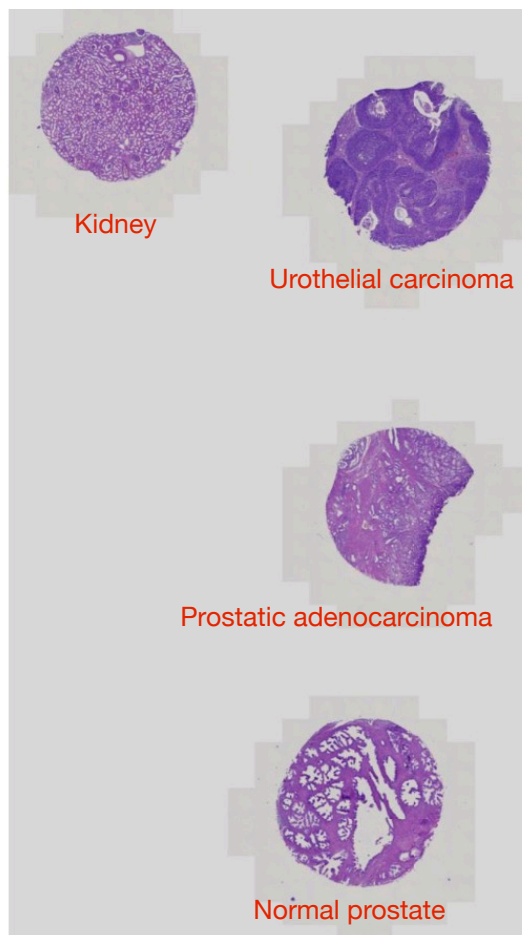
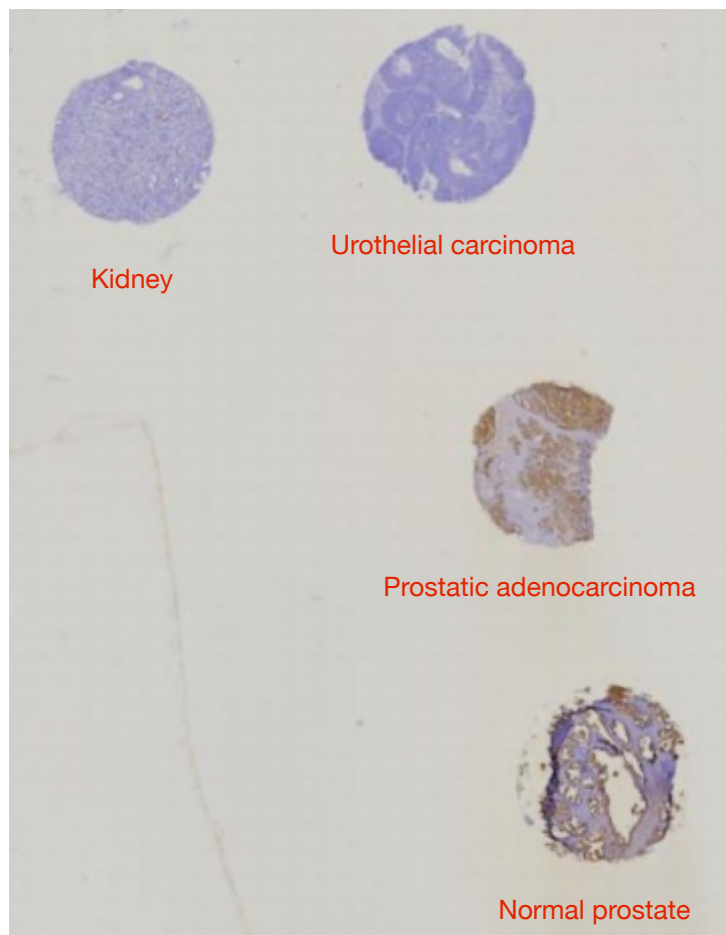




Prostate Specific Antigen (PSA)



HE staining in scanner view



PSA immunostaining, scanner view

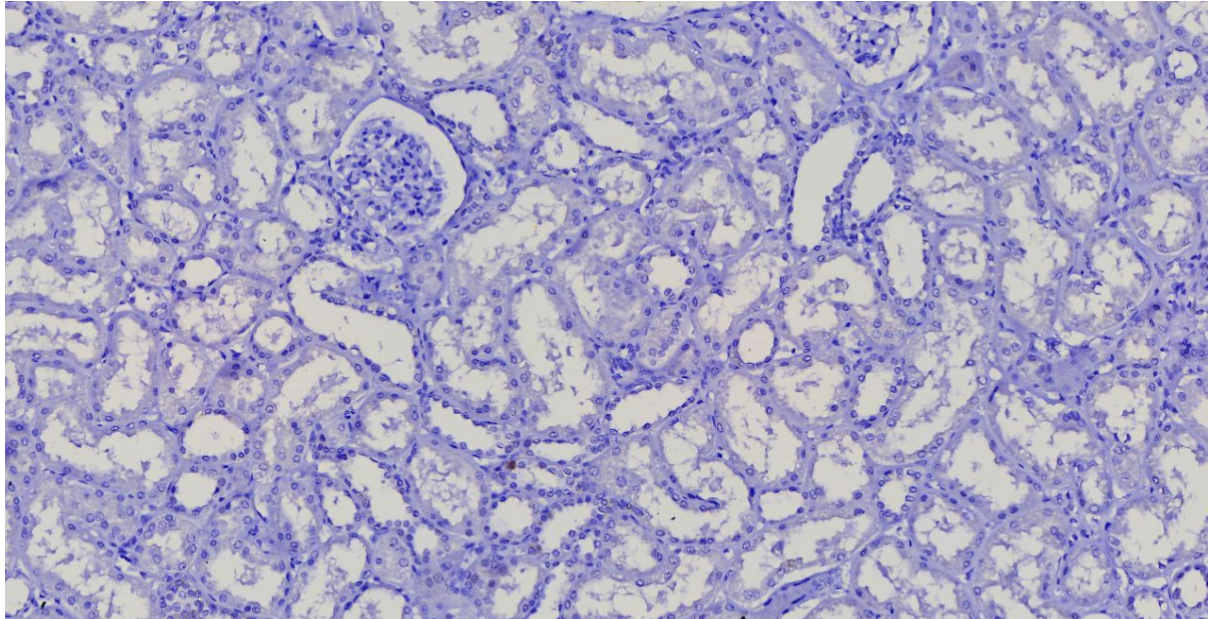
Provided sections in this run.

Kidney and urothelial carcinoma are negative stains for this marker while prostatic adenocarcinoma and normal prostate are positive stains for this marker.

Some degree of loss of integrity of section in normal prostatic tissue is observed in many participants and would not be judged as poor indicator if found in this range.

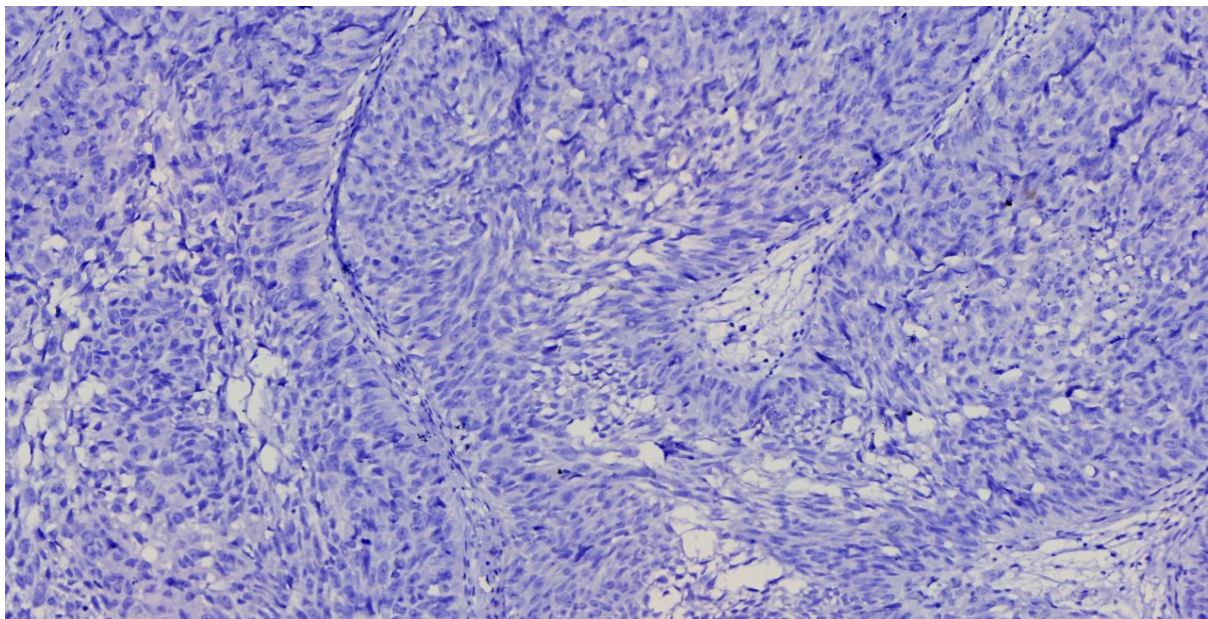


Kidney, PSA staining, 10x



No staining of any cell in the section

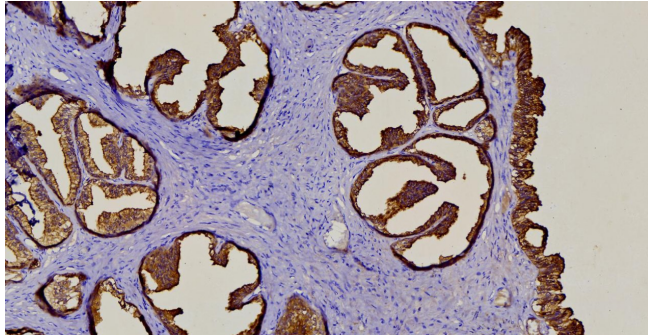
Urothelial carcinoma, PSA staining, 10x



No staining of any cell in the section

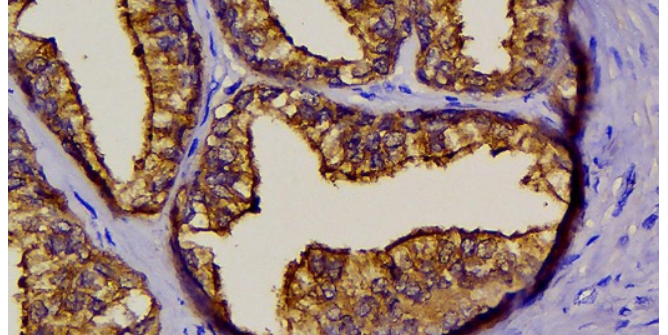


Normal prostate, PSA, 10x



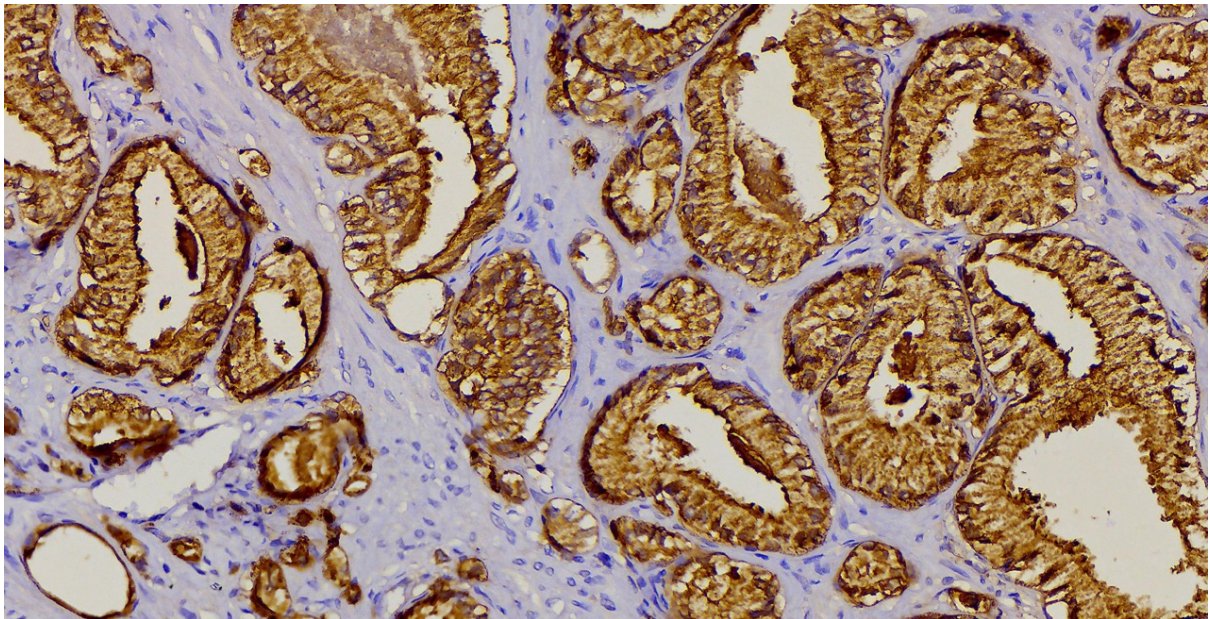
Diffuse staining of all the glands, stroma is unstained.

Normal prostate, PSA, 40x



Intense membranous staining of the epithelial cells and milder cytoplasmic staining.

Prostatic adenocarcinoma, PSA, 20x



Intense membranous staining of the atypical cells lining the neoplastic glands and milder cytoplasmic staining.



Protocol used by the top 3 in this run

Variable	1	2	3
Technique	AUTOMATED, VENTANA, BENCHMARK , GX	MANUAL	MANUAL
Clone	PSA	EP109	EP109
Vendor	VENTANA, 760-2506	BIOCARE MEDICALS CME 390AK	PathnSitu, MR1155
Format	RTU	CONCENTRATED	RTU
Batch/Year	G22198, 06.10.2020	2020 022920	R071155CA, NA
Expiry	20.09.2022	2022/03	04/22
Dewaxing temperature	76 DEGREES	55DEGREE	65°C
Retrieval	HIER	HIER	HIER
Enzyme	NA		NA
HIER	VENTANA	DECLOAKING CHANBER	Microwave oven
Peak T and Time	100 DEGREES ,4 MINUTES	110 DEGREE 20 MINS	110°C, 15 mins (5+5+5)
Peak Pressure and Time	NA	6PSI	NA
Retrieval Buffer	READY MADE CELL CONDITIONER 1, EDTA BASED	EDTA	Company provided, EnVision FLEX TARGET RETRIVAL SOLUTION.
pH	8.4	pH9	9.0
Blocking	NA	HYDROGENPEROXIDE	EnVision FLEX PEROXIDASE- BLOCKING REAGENT, 10 mins.
Wash sol	RTU, COMPANY BASED	TBS	Tris buffered saline with saponifying agent.
Dilution of RTU	NA		NA
Dilution of conc	NA	1:100	NA
Diluent	NA	DAVINCI GREEN	NA
Inc time of Primary	24 MINUTES	60 MINS	30 mins.
Detection	POLYMER BASED	POLYMER BASED	Polymer based system



Variable	1	2	3
Cat No	UV HRP, UNIV MULT, VENATANA, 760-500, H21101, 14.07.2023	MACH 1 BIOCARE MEDICALS	EnVision FLEX/ HRP, Dako, K8023, 41293161, NA, 2022-07-31.
Inc time of Sec	8 MINUTES	PROBE - 15 MINS HRP - 30 MINS	30 mins
Chro-substrate	8 MINUTES	5 MINS	8 mins.
Post-treatment	UV COPPER, 4 MINUTES		NA
Counterstain	HEAMATOXYLIN	HAEMOTOXYLIN	Harris hematoxylin.

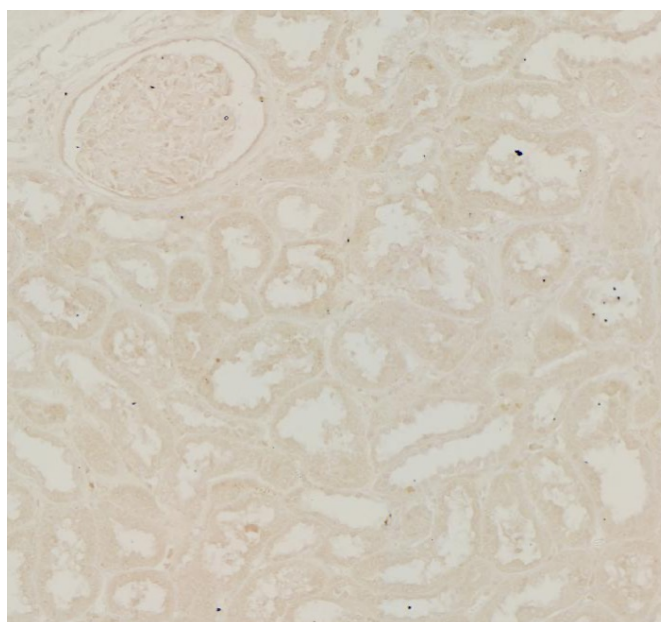


Fig.1 Background staining due to improper wash cycles in a participant using the same protocol as No.3 in top protocol (see Fig.2)

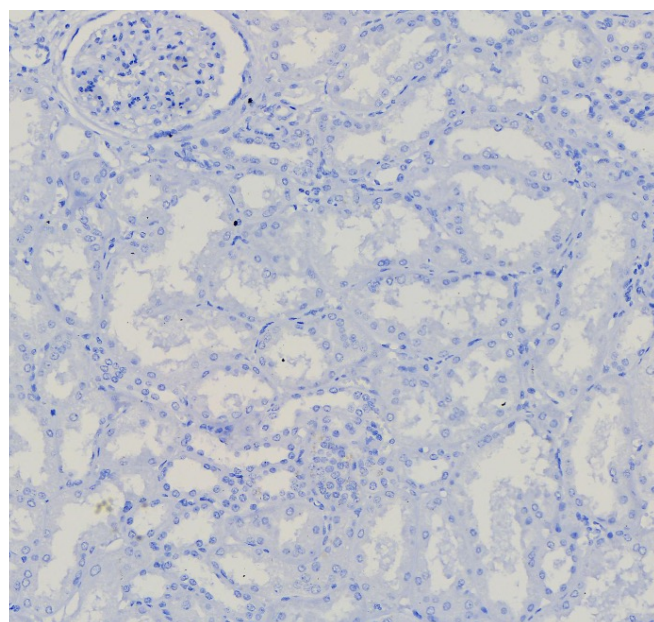


Fig. 2 No background staining in the participant using the protocol at number 3 in the table above

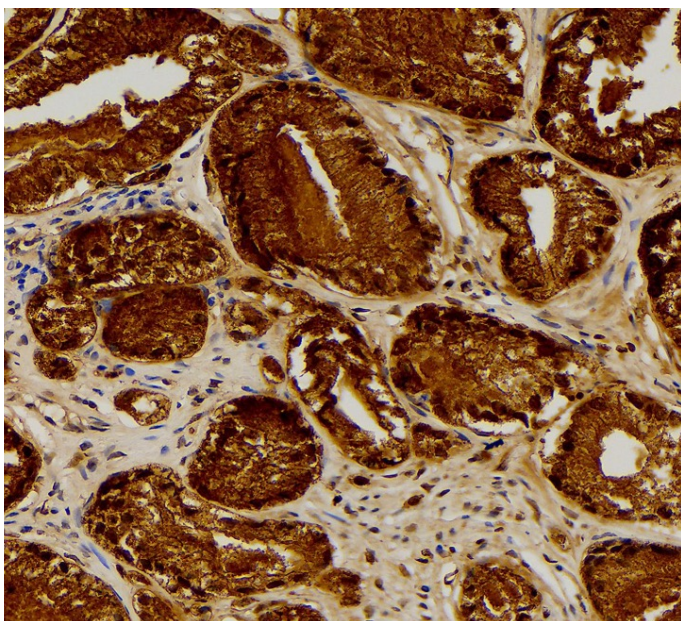


Fig.3 Loss of morphological details, intense staining and heavy background staining. The portal uses dilute RTU 20x. More dilution might be needed

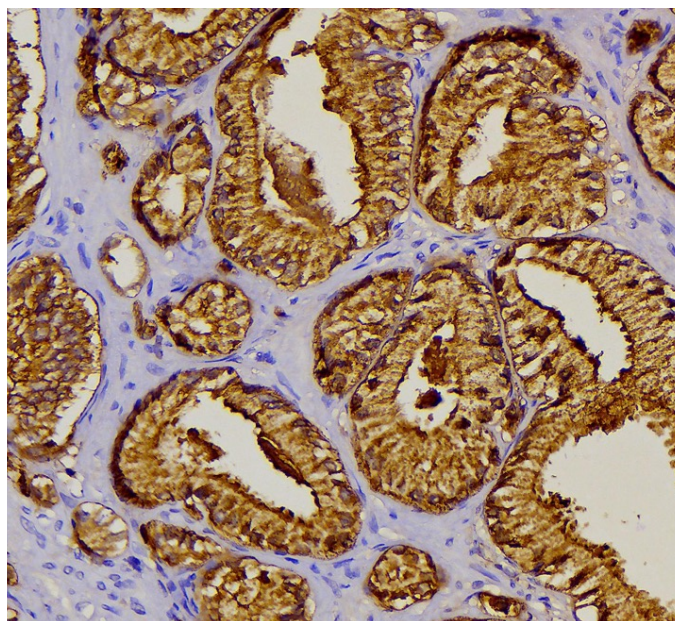


Fig.4 Optimum protocol setting produces right intensity without loss of morphological details and crisp background

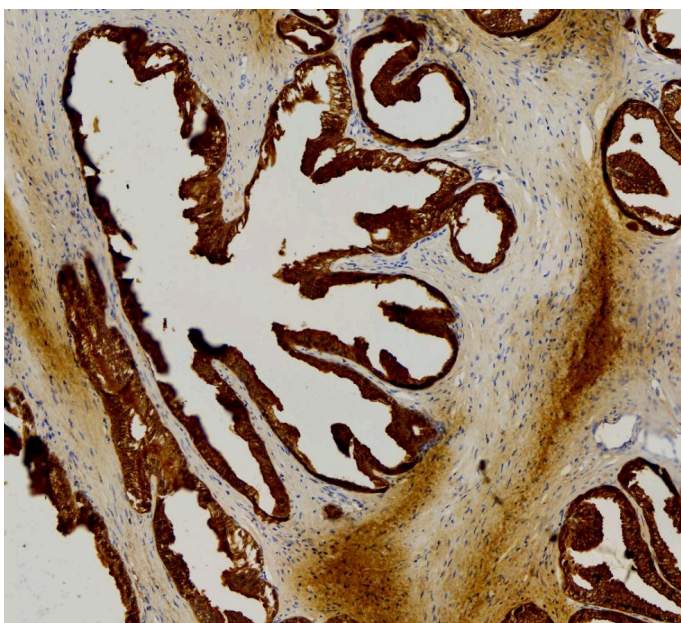


Fig.5 Same case as Fig.3 shows identical features in normal prostate

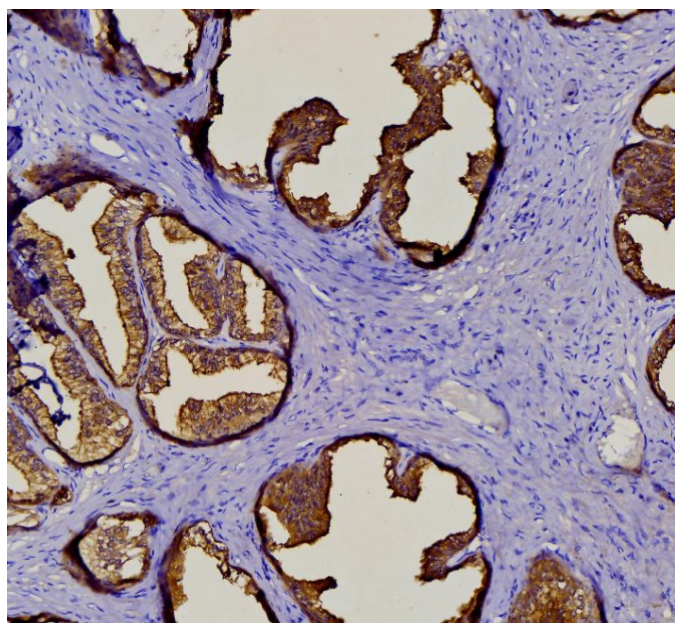


Fig.6 Same case as Fig.4