abcam

ab108338

Anti-ATG9A antibody [EPR2450(2)]

Anti-ATG9A antibody [EPR2450(2)] (ab108338) is a rabbit monoclonal antibody detecting ATG9A in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 80 publications

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR2450(2)
Purification technique	Affinity purification Protein A
Concentration	0.19 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/50
Notes	**For unpurified use 1/100 - 1/250.** Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000

Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50 - 1/100
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information ATG9A

Function	Phospholipid scramblase involved in autophagy by mediating autophagosomal membrane expansion (PubMed:22456507, PubMed:27510922, PubMed:29437695, PubMed:32513819, PubMed:32610138, PubMed:33106659, PubMed:33468622, PubMed:33850023). Cycles between the preautophagosomal structure/phagophore assembly site (PAS) and the cytoplasmic vesicle pool and supplies membrane for the growing autophagosome (PubMed:16940348, PubMed:22456507, PubMed:33106659). Lipid scramblase activity plays a key role in preautophagosomal structure/phagophore assembly by distributing the phospholipids that arrive through ATG2 (ATG2A or ATG2B) from the cytoplasmic to the luminal leaflet of the bilayer, thereby driving autophagosomal membrane expansion (PubMed:33106659). Also required to supply phosphatidylinositol 4-phosphate to the autophagosome initiation site by recruiting the phosphatidylinositol 4-kinase beta (PI4KB) in a process dependent on ARFIP2, but not ARFIP1 (PubMed:30917996). In addition to autophagy, also plays a role in necrotic cell death (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Stable for 12 months at -20°C

Notes

What is this antibody validated in?
Anti-ATG9A antibody [EPR2450(2)] (ab108338) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse, Rat samples.

What is the molecular weight of ATG9A?
Anti-ATG9A [EPR2450(2)] (ab108338) specifically detects a band for ATG9A (UniProt: Q7Z3C6) at a molecular weight of 94kDa.

Trusted by the scientific community
Anti-ATG9A [EPR2450(2)] (ab108338) was first used in a scientific publication in 2011 and has been cited over 80 times in peer-reviewed journals.

Reviewed by scientists
Anti-ATG9A [EPR2450(2)] (ab108338) has over 10 independent reviews from customers.

Specificity confirmed
The specificity of Anti-ATG9A antibody [EPR2450(2)] (ab108338) has been confirmed by Western blot testing in ATG9A Knockout HAP1 cells.

Other related products
We have a range of other formats of antibody clone [EPR2450(2)] also available for your convenience: ab108338, Alexa Fluor® 488 - ab206252, Alexa Fluor® 647 - ab206253, Carrier free - ab223528, ab309518, Carrier free - ab309519, APC - ab310808, PE - ab310890, Alexa Fluor® 594 - ab311672, Alexa Fluor® 568 - ab312947, Alexa Fluor® 750 - ab320894

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	ATG9A also known as APG9-like 1 or ATG9 autophagy related 9A is an important component in the autophagy machinery. This protein has a molecular weight of approximately 93 kDa. It is ubiquitously expressed in various tissues with higher expression seen in the heart and skeletal muscle. Mechanically ATG9A is essential for the trafficking of membranes necessary for autophagosome formation. It actively participates in membrane lipids' delivery from donor organelles which are critical for constructing autophagosomes.
Biological function summary	ATG9A facilitates the recycling of cellular materials through a process called autophagy. This protein acts with other autophagy-related proteins to form a complex essential for autophagosome elongation and closure. The ATG9A protein's dynamic movements between the Golgi apparatus and endosomes ensure proper membrane supply for autophagy. It coordinates with the ULK1 complex and various phospholipid-modifying enzymes which are necessary to regulate autophagic flux.

Pathways

	ATG9A plays an integral role in the mTOR signaling and MAPK pathways. Both pathways are essential for cellular responses to stress and nutrient availability. In these pathways ATG9A works closely with proteins like beclin 1 and ATG5. Inhibiting mTOR actively induces autophagy where ATG9A contributes to membrane recruitment and elongation of the autophagosomes facilitating the clearance of damaged organelles and proteins.
Associated diseases and disorders	ATG9A shows a significant link to neurodegenerative diseases such as Parkinson's and Alzheimer's disease. The dysfunction of autophagic pathways due to altered ATG9A function leads to the accumulation of damaged proteins and organelles. This event contributes to the pathogenesis of such neurodegenerative diseases. Moreover ATG9A closely associates with proteins like LAMP2 whose mutations similarly lead to defective autophagic processes resulting in the accumulation of unprocessed cellular debris in these disorders.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

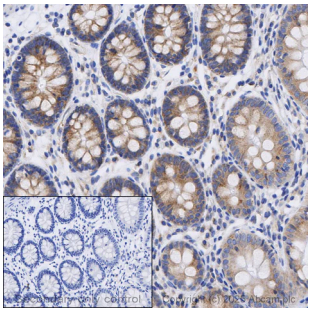
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

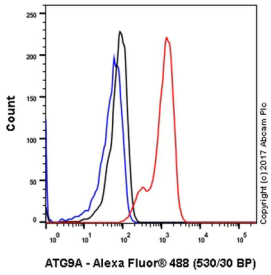
14 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human colon using rabbit Anti-ATG9A antibody

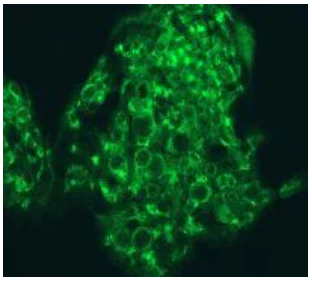
Immunohistochemical analysis of formalin fixed paraffin embedded human colon labelling ATG9A with ab108338 at a concentration of 0.5µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins. ab108338 anti-ATG9A antibody [EPR2450(2)] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.
Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual).



Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Flow Cytometry (Intracellular) staining using rabbit Anti-ATG9A antibody

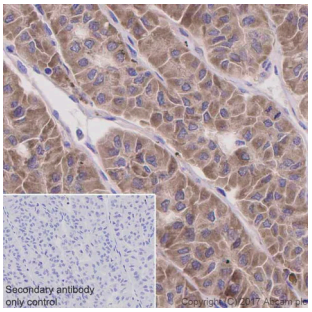
Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ATG9A with purified ab108338 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 100% Methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ATG9A antibody

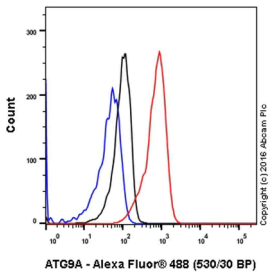
Unpurified ab108338 at 1/50 dilution, staining ATG9A in HepG2 (Human hepatocellular carcinoma epithelial cell) cells by Immunofluorescence.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ATG9A antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human thyroid carcinoma tissue sections labeling ATG9A with Purified ab108338 at 1:50 dilution (4.12 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

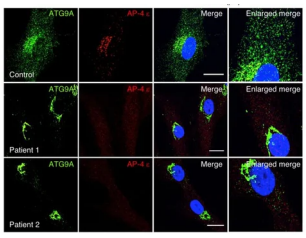


Flow Cytometry (Intracellular) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Flow Cytometry (Intracellular) staining using rabbit Anti-ATG9A antibody

Unpurified ab108338 staining ATG9Ain the human cell line HepG2 (human hepatocellular carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/40. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.
Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



De Pace, R. et al PLoS Genet. 2018 Apr 26;14(4):e1007363. doi: 10.1371/journal.pgen.1007363. eCollection 2018 Apr Reproduced under the Creative Commons license https://creativecommons.org/publicdomain/zero/1.0/

Immunocytochemistry/ Immunofluorescence - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

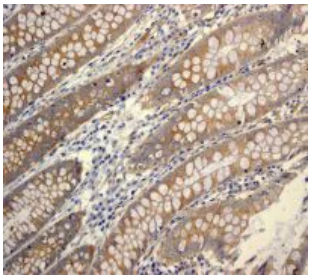
ATG9A Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ATG9A antibody

Accumulation of ATG9A at the TGN of AP-4 µ4 mutant patient fibroblasts.
Skin fibroblasts were from one control individual and two patients homozygous for mutations in the AP4M1 gene encoding AP-4 µ4.

Co-immunostaining for endogenous ATG9A (green) and AP-4 ε (red) (B) or GM130 of the fibroblasts.

ATG9A is detected using ab108338.

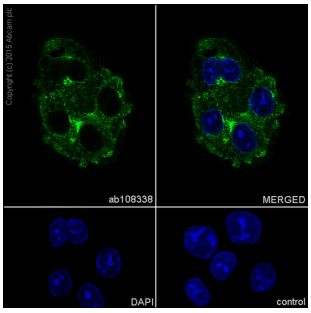
(From Figure 4B of De Pace et al)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ATG9A antibody

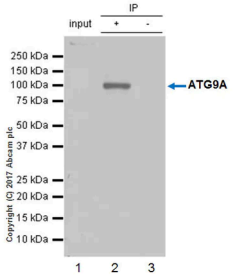
Unpurified ab108338, at 1/100, staining ATG9A in paraffin-embedded Human colon tissue by Immunohistochemistry.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ATG9A antibody

Immunocytochemistry/ Immunofluorescence analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ATG9A with Purified ab108338 at 1:100 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. ab150077 Goat anti rabbit IgG (Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



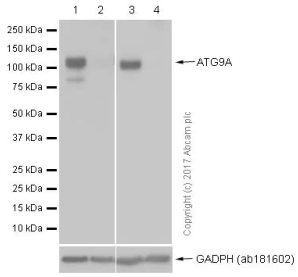
Immunoprecipitation - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ab108338 (purified) at 1:20 dilution (2µg) immunoprecipitating ATG9A in HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate.

Lane 1 (input): HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab108338 & HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab108338 in HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution. No band in input lane is due to the boiled lysates
Blocking and diluting buffer: 5% NFDM/TBST.
All lanes:
Immunoprecipitation - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

Predicted band size: 94 kDa



Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Western blot staining using rabbit Anti-ATG9A antibody

We suggest not to boil the sample after lysis.
Blocking and diluting buffer: 5% NFDM/TBST
Exposure time:
Left image: 5 seconds
Right image: 2 seconds

All lanes:
Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/10000 dilution

Lane 1:
293 (Human embryonic kidney epithelial cell) whole cell lysate prepared in non-boiled method at 15 µg

Lane 2:
293 (Human embryonic kidney epithelial cell) whole cell lysate prepared in boiled method at 15 µg

Lane 3:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate prepared in non-boiled method at 15 µg

Lane 4:

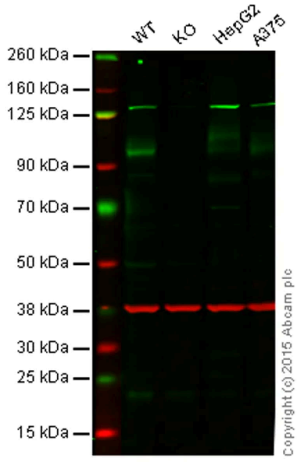
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate prepared in boiled method at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 94 kDa

Observed band size: 100 kDa



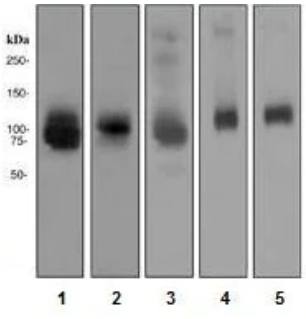
Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Western blot staining using rabbit Anti-ATG9A antibody

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: ATG9A knockout HAP1 cell lysate (20 µg)
Lane 3: HepG2 cell lysate (20 µg)
Lane 4: A375 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab108338 observed at 100 and 130 kDa. Red - loading control, ab8245, observed at 37 kDa.

Unpurified ab108338 was shown to specifically react with ATG9A when ATG9A knockout samples were used. Wild-type and ATG9A knockout samples were subjected to SDS-PAGE. ab108338 and ab8245 (loading control to GAPDH) were diluted 1/1000 and 1/2000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10,000 dilution for 1 h at room temperature before imaging.
All lanes:
Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

Predicted band size: 94 kDa



Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

All lanes:
Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/1000 dilution

Lane 1:
HepG2 cell lysate at 10 µg

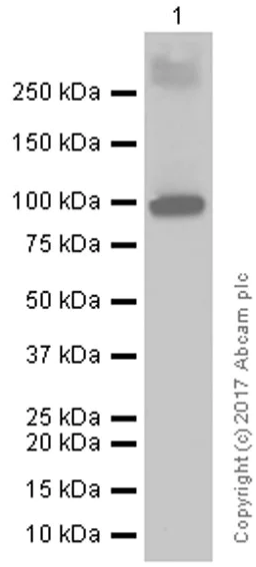
Lane 2:
293T cell lysate at 10 µg

Lane 3:
A375 cell lysate at 10 µg

Lane 4:
Mouse brain cell lysate at 10 µg

Lane 5:
Rat brain cell lysate at 10 µg

Predicted band size: 94 kDa



Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Western blot staining of Mouse spinal cord lysates using rabbit Anti-ATG9A antibody

Blocking and diluting buffer: 5% NFDM/TBST.
The lysates are boiled.

All lanes:
Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/2000 dilution

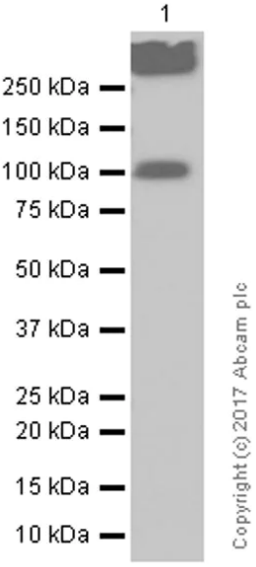
All lanes:
Mouse spinal cord lysates at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 103 kDa, 107 kDa, 110 kDa, 134 kDa, 137 kDa, 14 kDa, 169 kDa, 190 kDa, 20 kDa, 21 kDa, 23 kDa, 24 kDa, 25 kDa, 28 kDa, 30 kDa, 33 kDa, 42 kDa, 43 kDa, 47 kDa, 51 kDa, 53 kDa, 55 kDa, 74 kDa, 83 kDa, 90 kDa, 94 kDa, 96 kDa, 99 kDa

Observed band size: 103 kDa, 120 kDa, 14 kDa, 150 kDa, 169 kDa, 175 kDa, 18 kDa, 193 kDa, 21 kDa, 28 kDa, 32 kDa, 32-42 kDa, 35 kDa, 42 kDa, 44 kDa, 45 kDa, 47 kDa, 51 kDa, 53 kDa, 55 kDa, 90 kDa



Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338)

ATG9A Western blot staining of Rat brain lysates using rabbit Anti-ATG9A antibody

Blocking and diluting buffer: 5% NFDM/TBST.
The lysates are boiled.

All lanes:
Western blot - Anti-ATG9A antibody [EPR2450(2)] (ab108338) at 1/2000 dilution

All lanes:
Rat brain lysates at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 42 kDa, 47 kDa, 53 kDa, 76 kDa, 82 kDa, 94 kDa

Observed band size: 50-70 kDa, 53 kDa, 72 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.