Electron Microscopy in BIOCEV
2020

External payment incures extra overhead/administration fee 17.65% and VAT

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Pricelist is only orientational and all fees depend on the number of prepared samples and on the chosen methods that the best fit your project requirements.

CHEMICAL FIXATION		
Standard sample preparation for TEM	90 Kč / block (max 1mm3)	2.5 % GA in 0.1M buffer 1% OsO4 Alcohol for dehydratation Resin for embedding
Assistance	1.600 Kč / run (10 samples max)	8 hours of labour process
Ultrathin sectioning	150 Kč / block	Glass and diamond knives Cover glass Toluidine blue Instrument running cost
Ultrathin sectioning assistance	200 Kč / hour	
Section contrasting with uranyl acetate (up to 10 grids)	200 Kč / run	
Section contrasting with lead citrate (up to 10 grids)	200 Kč / run	
Application of formvar (up to 20 grids)	200 Kč / run	
Extra work for more complicated samples	200 Kč / hour	
Extra material:		
2% OsO4	130 Kč	2 mL
4% OsO4	140 Kč	2 mL
25% glutaraldehyde	80 Kč	10 mL
50% glutaraldehyde	115 Kč	10 mL
16% formaldehyde	80 Kč	10 mL
Au grids (200 mesh)	30 Kč	Each
Cu grids (200 mesh)	7 Kč	Each
Cu grids (400 mesh)	9 Kč	Each
Ni grids (200 mesh)	9 Kč	Each
Epon EmBed812 resin	80 Kč	50 mL

AUTOMATIC FREEZE SUBSTITUTION (AFS)		
AFS samples for ultrastructure	2.600 Kč / run (up to 10 samples) 3.050 Kč / run (11-20 samples)	Acetone Epon EmBed 812 resin 2% OsO4 Liquid nitrogen Assistance
AFS samples for immunolabeling	4.600 Kč / run (up to 10 samples)	Acetone Lowicryl resin Liquid nitrogen Assistance

HIGH PRESSURE FREEZING (HPM) – cell suspension		
Gold membrane	220 Kč / membrane	
3 mm carrier	230 Kč / 2 carriers	
6 mm carrier	400 Kč / 2 carriers	
Cryprotectant (bovine serum albumine)	90 Kč / mL	
Liquid nitrogen	580 Kč / 30 samples or ½ day freezing	
Assistance	600 Kč / 30 samples or ½ day freezing	
HIGH PRESSURE FREEZING (HPM) – cell monolayer		
3 mm sapphire with finder grid	600 Kč / sample	Sapphire + holder Instrument running costs
6 mm sapphire in brown plates	730 Kč / sample	Sapphire Spacer Carrier Instrument running costs
6 mm sapphire in black plate	650 Kč / sample	Sapphire Spacer Carrier Instrument running costs
Cryoprotectant (bovine serum albumine)	90 Kč / mL	
Liquid nitrogen	580 Kč / 30 samples or ½ day freezing	
Assistance	600 Kč / 30 samples or ½ day freezing	

CRITICAL POINT DRYING (CPD)		
Chemical fixation before CPD (obligatory)	200 Kč / hr	Based on the number of hours
CPD 1 run	750 Kč	

HIGH VACUUM COATER	
Instrument run-time (carbon coating; sputtering)	300 Kč / hr
Glow discharge	150 kč / run
Sputtering (platinum or gold)	4 Kč / nm

MICROSCOPES		
FIB-SEM	950 Kč / hr	Including assistance
SEM	800 Kč /hr	Including assistance
TEM	300 Kč / hr	Without assistance

IMMUNO-LABELLING: Secondary Antibodies (Jackson Immunoresearch)	
Goat-anti-RAT 6nm	17 kč / 1uL
Goat-anti-RABBIT 6nm	11 Kč / 1uL
Goat-anti-RABBIT 12nm	7 Kč / 1 uL
Goat-anti-MOUSE 6nm	11 Kč / 1uL
Goat-anti-MOUSE 12nm	12 Kč / 1uL

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EXAMPLES: sample preparation

1) **Chemical fixation:** You would like us to prepare for you 5 samples with chemical fixation procedure. The final resin-embedded samples would be cut in our facility onto formvar-carbon-coated copper grids to support the ultrathin sections when observing in TEM microscope. Other option is to prepare the sample for FIB-SEM microscope. The pricing would be as followed:

Procedure	Quantity	Price / unit	Total in CZK (no VAT)
Chemical fixation	5 samples in duplicates	90	900
Assistance	1 set	1.600	1 600
Sectioning	5 samples	150	750
Sectioning assistance	5 samples = 5 hours	200	1 000
Sectioning on Cu grids with formvar	1 formvar cover slide	200	200
Carbon coating of formvar grids	1 run	150	150
Cu grids	15	7	105
Glow discharge	1 run	150	150
Section contrasting with uranyl acetate	15 grids (1,5 hr)	200/ hr	300
Section contrasting with lead citrate	15 grids (1,5 hr)	200 /hr	300
TOTAL			**5455**

2) **High pressure freezing of monolayers**: You would like us to prepare for you 2 samples (control and treated cells) for **ultrastructure** detection in cancer monolayer cells using freezing these cells. In this procedure, no chemical fixation is needed. The samples would be processed up to final resin embedding and sectioned for TEM microscope.

   We recommend you to culture your cells on sapphire disk for 24 – 48h till almost fully confluent. Sapphire disks are provided by our facility. It is better to have more sapphire disk covered with cells – e. g. 5 disks for controls and 5 for treated cells (totally 10 sapphires). Other option is to culture your cells on aclar disk till fully confluent. Aclar disks are provided by our facility.

   The cells will be processed through HPF – high pressure freezing (using liquid nitrogen and high pressure), AFS – automatic freeze substitution (that takes cells from frozen condition up to room temperature condition), and finally, ultrathin cutting of the room-temperatured cells. The sections are now prepared for TEM microscope.

   Other option is to prepare the samples this way for FIB-SEM microscope (continue to Example 4.).

Procedure	Quantity	Price / unit	Total in CZK (no VAT)
HPF: 3 mm sapphire	10	600	6 000
HPF: cryoprotectant	1	90	90
HPF: liquid nitrogen	1	580	580
HPF: Assistance	1	600	600
AFS: sample for ultrastructure including operator time	1 run (10 samples)	2600	2600
Sectioning	10 samples	150	1500
Sectioning assistance	10 samples = 10 hours	200	2000
Sectioning on Cu grids with formvar	2 formvar cover slide	200	400
Carbon coating of formvar grids	1 run	150	150
Glow discharge	1 run	150	150
Cu grids	30	7	210
Section contrasting with uranyl acetate	30 grids (3 hrs)	200 /hr	600
Section contrasting with lead citrate	30 grids (3 hrs)	200 / hr	600
TOTAL			**15480**

3) **High pressure freezing of suspensions / tissues:** You would like us to prepare for you 2 samples (control and treated cells) for **ultrastructure** detection in suspension cells or tissue cultures using freezing these cells/tissues. In this procedure, no chemical fixation is needed. The samples would be processed up to final resin embedding and sectioned for TEM microscope. Other option is to prepare the samples this way for FIB-SEM microscope.

   You will bring us your live (un-fixed) suspension cells. The sample will be centrifuged with/without cryoprotectant (bovine serum albumin or other) and placed into special freezing holder (carrier or membrane). Additional cryoprotectant could be added to cells before freezing. The cells will be processed through HPF – high pressure freezing (using liquid nitrogen at high pressure), AFS – automatic freeze substitution (that takes cells from frozen condition up to room temperature condition), and finally, ultrathin cutting of the room-temperatured samples. The sections are now prepared for TEM microscope.

   Other option is to prepare the samples this way for FIB-SEM microscope (continue to example 4.).

Procedure	Quantity	Price / unit	Total in CZK (no VAT)
HPF: 3 mm carriers	10	230	2300
HPF: cryoprotectant	1	90	90
HPF: liquid nitrogen	1	580	580
HPF: assistance	1	600	600
AFS: sample for ultrastructure including operator time	1 run (10 samples)	2.600	2600
Sectioning	10 samples	150	1500
Sectioning Assistance	10 samples	200	2000
	= 10 hours		
Sectioning on Cu grids with formvar	1 formvar cover slide	200	200
Carbon coating of formvar grids	1 run	150	150
Cu grids (3 per 1 sample)	30	7	210
Glow discharge	1 runs	150 / run	150
Section contrasting with uranyl acetate	30 grids (3 hrs)	200	600
Section contrasting with lead citrate	30 grids (3 hrs)	200	600
TOTAL			**11580**

4) **Sample preparation for FIB-SEM** (for initial steps, see Example 2. or Example 3.)

When samples go **through HPF/FS** procedure and are embedded for FIB-SEM, the next step is platinum or gold sputtering for high vacuum detection (25 nm per sample).

Based on this, the **orientational pricing** (not included EM detection) would be:

Procedure	Quantity	Price / unit	Total in CZK (no VAT)
HPF: 3 mm carriers	10	230	2300
HPF: cryoprotectant	1	90	90
HPF: liquid nitrogen	1	580	580
HPF: assistance	1	600	600
AFS: sample for ultrastructure including operator time	1 run (10 samples)	2.600	2600
High vacuum coater - sputtering	1 run	150	150
Platinum sputtering	25 nm	4 Kč / nm	100
TOTAL			**6420**

5) **Immunogold labelling:** Your samples could also be prepared for immunogold labelling. The samples (monolayers or suspensions) are prepared either through chemical fixation (post-embedding immunolabelling) or through high pressure freezing/freeze substitution process. Finally, the samples are embedded in resins suitable for immunolabelling. The resin-embedded samples would be cut in our facility onto formvar-carbon-coated nickel (or gold) grids to support the ultrathin sections when observing in TEM microscope.

   Immunogold labelling is not a standard sample preparation, it requires more time and co-operation with our facility members. Therefore, the pricing list is not included. Please, contact us for more details.

6) Critical Point Drying (CPD) for SEM imaging: You would like to prepare several samples for SEM imaging. Your samples would be chemically fixed (aldehydes, osmium contrastation /optional/, dehydratation in ethanl series) and processed using Critical Point Drying with acetone). CPD is method that preserves the surface structure of a specimen which could otherwise be damaged due to surface tension when changing from the liquid to gaseous state. The orientation price would be as followed:

Procedure	Quantity	Price / unit	Total in CZK (no VAT)
Chemical fixation	1	600	600
Critical Point Dryinig	1	750	750
Carbon coating	1	150	150
TOTAL			**1500**