

Electron Microscopy - BIOCEV 2021

External payment - charges incurred: extra overhead/administration fee 17.65% and VAT

Assistance and instrument costs are subsidized by Czech Biolmaging Projects:
LM2018129 and CZ.02.1.01/0.0/0.0/18_046/0016045, funded by MEYS CR.

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 (1mm³ max)	90 Kč / block	2.5 % GA in 0.1M buffer 1% OsO ₄ Alcohol for dehydration Resin for embedding
Assistance (10 samples max)	1.600 Kč / run	8 hours of labour process
Ultrathin sectioning	150 Kč / hr	Glass and diamond knives Cover glass Toluidine blue Instrument running cost
Ultrathin sectioning assistance	200 Kč / hr	
Section contrasting with uranyl acetate (up to 10 grids)	200 Kč / run	
Section contrasting with lead citrate (up to 10 grids)	200 Kč / run	
Negative staining (uranyl acetate or PTA staining)	200 Kč / run	
Application of formvar (up to 20 grids)	200 Kč / hr	
Extra work for more complicated samples	200 Kč / hr	
Extra material		
MatTek dish (sterile) with finder grid	213 Kč	Each
2% OsO₄ aqueous solution	110 Kč	2 mL
4% OsO₄ aqueous solution	128 Kč	2 mL
25% glutaraldehyde	90 Kč	10 mL
50% glutaraldehyde	115 Kč	10 mL
16% formaldehyde	80 Kč	10 mL
Au grids (200 mesh)	30 Kč	Each
Cu grids (100 mesh)	6 Kč	Each
Cu grids (200 mesh)	7 Kč	Each
Cu grids (400 mesh)	7 Kč	Each
Ni grids (100 mesh)	8 Kč	Each
Ni grids (200 mesh)	9 Kč	Each
Cu SLOT Grid 2 x 0.5mm	8 Kč	Each
4% Uranyl acetate aqueous solution	150 Kč	10mL
Epon EmBed812 resin	80 Kč	45 mL

FREEZE SUBSTITUTION (FS)

FS samples for ultrastructure (monolayers)	2596 Kč / run	chemicals costs, liquid nitrogen, assistance
FS samples for ultrastructure (suspensions)	3266 Kč / run	chemicals costs, liquid nitrogen, assistance
FS samples for immuno (up to 10 samples)	4388 Kč / run	chemicals costs, liquid nitrogen, assistance

HIGH PRESSURE FREEZING (HPF) – cell suspension

Gold membrane	214 Kč	
3 mm carrier sandwich (type A+A)	236 Kč / 2 carriers	
3 mm carrier sandwich (type A+B)	231 Kč / 2 carriers	
6 mm carrier sandwich (type A+A)	380 Kč / 2 carriers	
6 mm carrier sandwich (type A+B)	370 Kč / 2 carriers	
Cryoprotectant (bovine serum albumine)	106 Kč / mL	
Cryoprotectant (hexadecene)	20 Kč / run	
Liquid nitrogen	670 Kč	30 samples or ½ day freezing
Assistance	600 Kč	30 samples or ½ day freezing

HIGH PRESSURE FREEZING (HPF) – cell monolayer

3 mm sapphire with finder grid	732 Kč / sample	sapphire, sandwich, instrument running costs
3 mm sapphire	411 Kč / sample	sapphire, sandwich, instrument running costs
6 mm sapphire	642 Kč / sample	sapphire, sandwich, instrument running costs
Cryoprotectant (bovine serum albumine)	106 Kč / mL	
Liquid nitrogen	670 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 (sputtering)	300 Kč / hr	
Carbon coating	150 Kč / run	
Glow discharge	150 Kč / run	
Sputtering (platinum or gold)	4 Kč / nm	

MICROSCOPES and TRAINING

FIB-SEM	950 Kč / hr	Including assistance
SEM	800 Kč / hr	Including assistance
TEM	300 Kč / hr	Without assistance
TEM	500 Kč / hr	Including assistance
TEM training (2 x 3hr sessions)	free	
TEM training (user's samples are imaged)	300 Kč / hr	Including assistance

IMMUNO-LABELLING: Secondary Antibodies (Jackson Immunoresearch)

Goat-anti-RAT 6nm	17 Kč / uL
Goat-anti-RABBIT 6nm	11 Kč / uL
Goat-anti-RABBIT 12nm	7 Kč / uL
Goat-anti-MOUSE 6nm	11 Kč / uL
Goat-anti-MOUSE 12nm	12 Kč / uL

ACKNOWLEDGEMENT: We acknowledge Imaging Methods Core Facility at BIOCEV, institution supported by the MEYS CR (Large RI Project LM2018129 Czech-BioImaging) and ERDF (project No. CZ.02.1.01/0.0/0.0/18_046/0016045) for their support with obtaining imaging data presented in this paper.



Electron microscopy IMCF - BIOCEV May 2021

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 observed 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	90.00	900.00
Assistance	1 set	1.600	1600.00
Sectioning	5 samples	150.00	750.00
Sectioning assistance	5 samples = 5 hours	200.00	1000.00
Sectioning on Cu grids with formvar	1 formvar cover slide	200.00	200.00
Carbon coating of formvar grids	1 run	150.00	150.00
Cu grids	15	7.00	105.00
Glow discharge	2 runs	150.00	300.00
Section contrasting with uranyl acetate	15 grids (1,5 hr)	200/ hr	300.00
Section contrasting with lead citrate	15 grids (1,5 hr)	200 /hr	300.00
TOTAL			5605.00

- 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 confluency of around 70-80%. 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 foil till fully confluent. Aclar foil are provided by our facility.

The cells will be processed through HPF – high pressure freezing (using liquid nitrogen and high pressure), FS – freeze substitution (that takes cells from frozen condition up to room temperature condition), and finally, ultrathin cutting of the room-temperated 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 sapphires in sandwich	10	732	7320
HPF: cryoprotectant	1	106	106
HPF: liquid nitrogen	1	670	670
HPF: Assistance	1	600	600
FS: sample for ultrastructure including operator time	1 run	2596	2596
Sectioning	10 samples	150	1500
Sectioning assistance	10 samples	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	4 run	150	600
Cu grids (3grids per sample)	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			17352

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), FS – freeze substitution (that takes cells from frozen condition up to room temperature condition), and finally, ultrathin cutting of the room-temperated 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 sandwich (typo A+A)	10	236	2360
HPF: cryoprotectant	1	106	106
HPF: liquid nitrogen	1	670	670

HPF: assistance	1	600	600
FS: sample for ultrastructure including operator time	1 run	3266	3266
Sectioning	10 samples	150	1500
Sectioning Assistance	10 samples	200	2000
Sectioning on Cu grids with formvar	2 formvar cover slide	200	400
Carbon coating of formvar grids	1 run	150	150
Cu grids (3 per 1 sample)	30	7	210
Glow discharge	4 runs	150 / run	600
Section contrasting with uranyl acetate	30 grids (3 hrs)	200	600
Section contrasting with lead citrate	30 grids (3 hrs)	200	600
TOTAL			13062

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 of metal 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 sandwich (typo A+A)	10	236	2360
HPF: cryoprotectant	1	106	106
HPF: liquid nitrogen	1	670.00	670.00
HPF: assistance	1	600.00	600.00
FS: sample for ultrastructure including operator time	1 run	3266.00	3266.00
High vacuum coater - sputtering	1 run	150.00	150.00
Platinum sputtering	25 nm	4 Kč / nm	100
TOTAL			7252

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 fixation /optional/, dehydration in ethanol/acetone 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 orientational price would be as followed:

Procedure	Quantity	Price / unit	Total in CZK (no VAT)
Chemical fixation (3hrs)	3	200	600
Critical Point Dryinig	1	750	750
Carbon coating	1	150	150
TOTAL			1500

Electron microscopy IMCF - BIOCEV May 2021