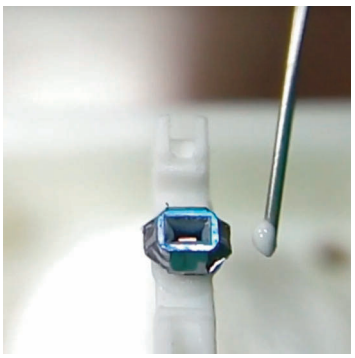
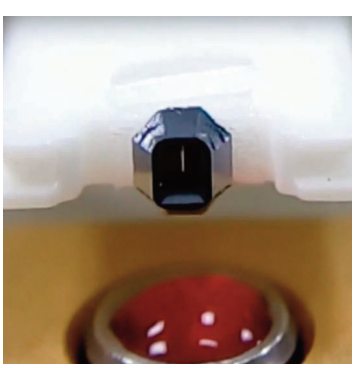
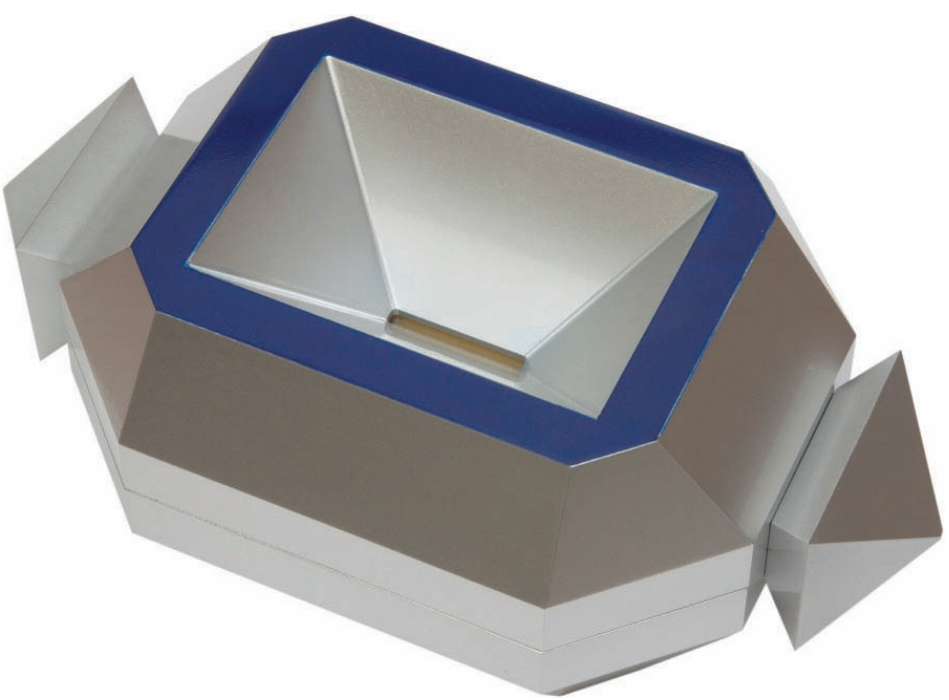
an innovative enclosed specimen holder
for Liquid TEM



Wet “Liquid” TEM Kit

K-kit – Silicon-based Micro Channel Device





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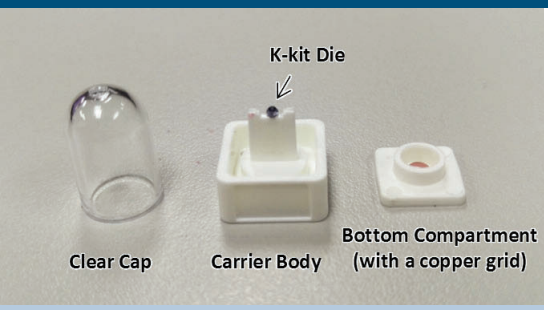

Why K-Kit?

K-kit Meets All Needs for Liquid TEM

1 Native State in Liquid

- **Available with undiluted solution.**
- **Preserve the original morphology and physical state in liquid**

2 In-situ Observation

- **Kinetic mechanism of metal growth or physicochemical reaction process in liquid can be in-situ observed with increased reaction time.**

3 Quantitative Analysis

- **Software of image recognition for nanoparticle size distribution analysis.**

4 Compatible with Versatile Microscopy Analyses

- **Applicable to TEM, FIB, and STEM.**
- **Available for EDX analysis.**
- **High resistance to most chemicals.**
- **Working temperature range from -40°C to 120°C.**

Patents being issued and publication:

US 7807979 B2
US 8969827 B2
Anal. Chem.2012, 84: 6312-6316

Electron Microscopy Sciences

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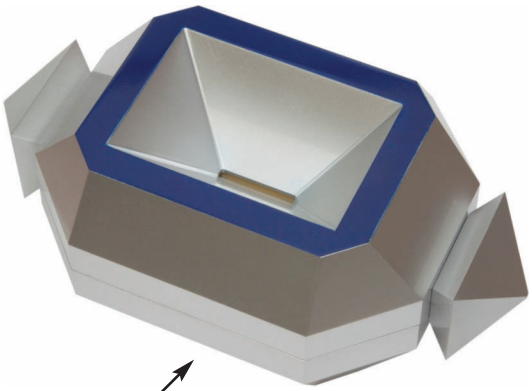
***or* stacie@ems-secure.com**
www.emsdiasum.com

K-Kit

A Specimen Holder for Liquid Sample Analysis in TEM



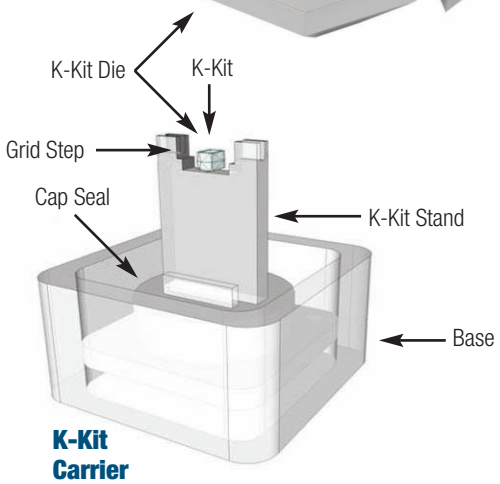
Overview

K-kits are sample holders designed to facilitate convenient TEM observation of liquid samples, allowing nano-objects, aggregates, and agglomerates (NOAAs) in liquid samples to be characterized.

With vacuum compatible sealing of liquids in electron-transmitting thickness, K-kits are micro reaction chambers for countless experiments in materials, chemical, and biological research.

Features

- **Applicable for most TEM holder brands**
- **Strong structural reliability under vacuum**
- **Sealing glue compatible to many solvents**
- **Disposable**
- **Free of cross-contamination**
- **Easy to use**

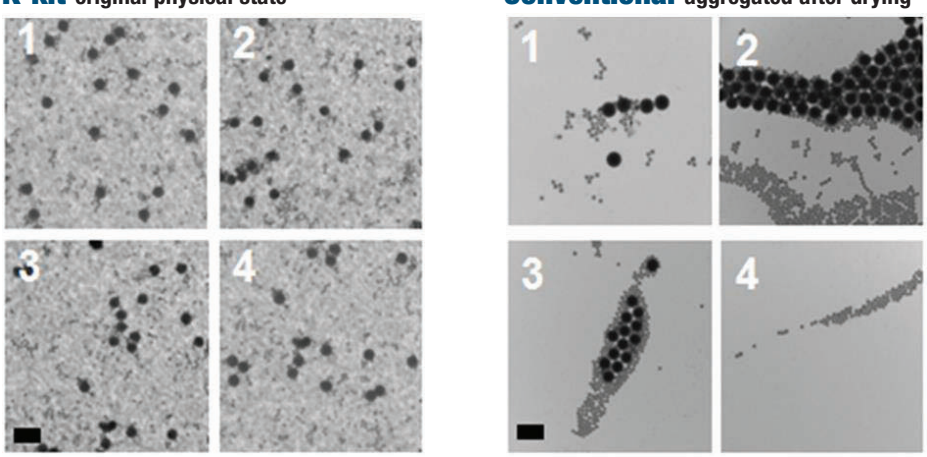


K-Kit Carrier

Sample Analysis Comparison

K-kit original physical state

Conventional aggregated after drying



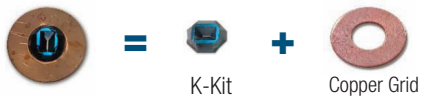
Images shown: NIST traceable polystyrene beads. Scale Bar 500nm.

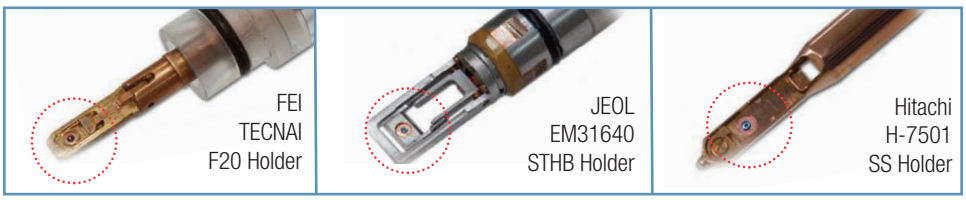
Physicochemical Parameters	K-kit	Cu-Grid
Composition	✓	✓
Size	✓	✓
Shape	✓	✓
Size Distribution	✓	Δ
Aggregation and Agglomeration *in liquid*	✓	X
Particle Concentration	✓	X
Liquid TEM Observation	✓	X

✓ = Good Δ = Case Dependent X = Not Available

K-kit Adaptability

Compatible with all kits of TEM Holders

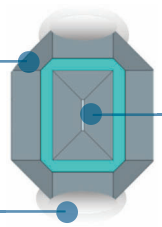




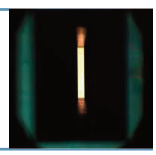
Strong Structural Reliability under Vacuum

Silicon Body (MEMS technology): Use a Si-based structure, ensuring strength and reliability. (MEMS, Micro-Electro-Mechanical Systems)

Torr Seal® Epoxy: A trusted and widely-used glue, suitable for high-vacuum systems. (Torr Seal®, a trade mark owned by Agilent Tech. Inc.)



Silicon Nitride Observation Window: Material intrinsically tough, durable to withstand drastic pressure changes.



Sealing glue compatible with many solvents

The following table shows the test results of Torr Seal Epoxy soaked in chemical solvents for 24 hours and then examined using FTIR (if dissolved), and visual observation (if dispersed).

	Water	PEG400	DMSO	Ethanol	0.1N HCl	0.1N KOH
Compatibility (FTIR)	✓	✓	✓	✓	✓	✓
	Hexane	**IPA**	**Methanol**	**DCM**	**THF**	**Acetone**
Compatibility (FTIR)	✓	✓	✓	X	X	X

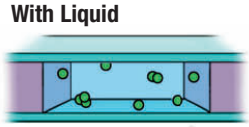
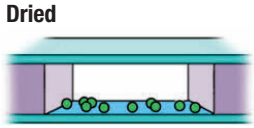
(FTIR, Fourier Transform Infrared Spectroscopy) ✓ = Compatible (FTIR not detected) X = Use with care (FTIR detected)

Wet and Thin Layer Mode of K-kit

The K-Kit can be used in either Wet Mode or Thin Layer Mode.

Wet Mode: The loaded liquid sample is sealed and imaged using TEM in the native liquid environment.

Thin Layer Mode: A patented liquid drying protocol preserves the original morphology and physical state of nanomaterials with improved imaging resolution.

Sample Preparation	Wet Mode	Thin Layer Mode
Inner Status of K-kit	**With Liquid** 	**Dried** 
Imaging Resolution	Good	Excellent
Gap Size (Considered)	300～500nm	2000～3000nm
Particle Size (Loadable)	10nm～300nm	3nm～2000nm
Particle Shape	Keeping original	Potentially, could be deformed.
Chemical Reduction or Potential Damage by Electron Energy	High	Low

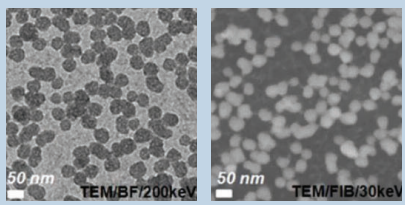
If making a Thin Layer (Dried) mode of K-kit, it's essential to keep both ends of the channel open to atmosphere, no need to do the channel gluing step.

Gap Height (um)	0.1	0.2	0.5	1.0	2
Wet Mode	●	●	◗	—	—
Thin Layer Mode	◗	●	●	●	●

EXAMPLES

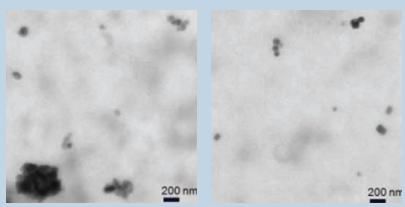
Characterizing NOAAs in liquid

Electronics



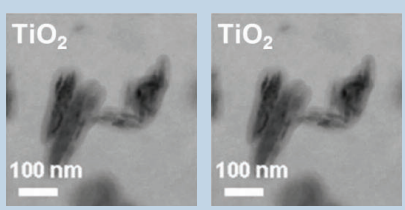
SiO_2 Nanoparticles in Polishing Slurry

Food & Beverage



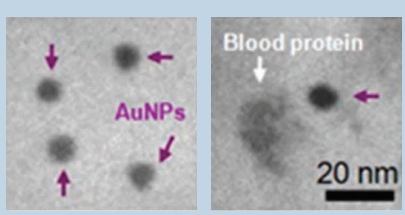
$CaCO_2$ Nanoparticle Additives in Milk

Cosmetics



TiO_2 and ZnO Nanoparticles in Sunscreen Lotion

Pharmaceuticals



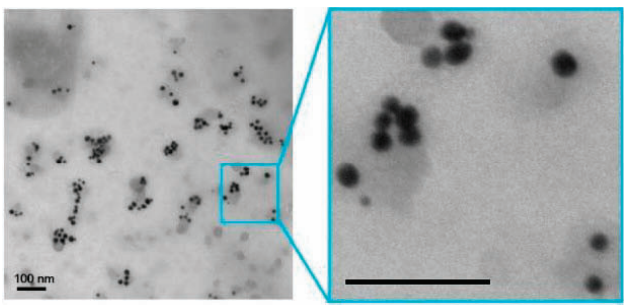
Gold Nanoparticles (AuNPs) in Blood

Electron Microscopy Sciences

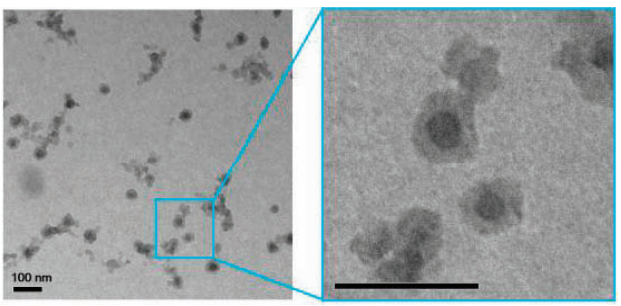
Liquid-TEM Observation in Nanopharmaceuticals

Applicable particle concentration for K-kit: 10^{11}~10^{14} particles/ml

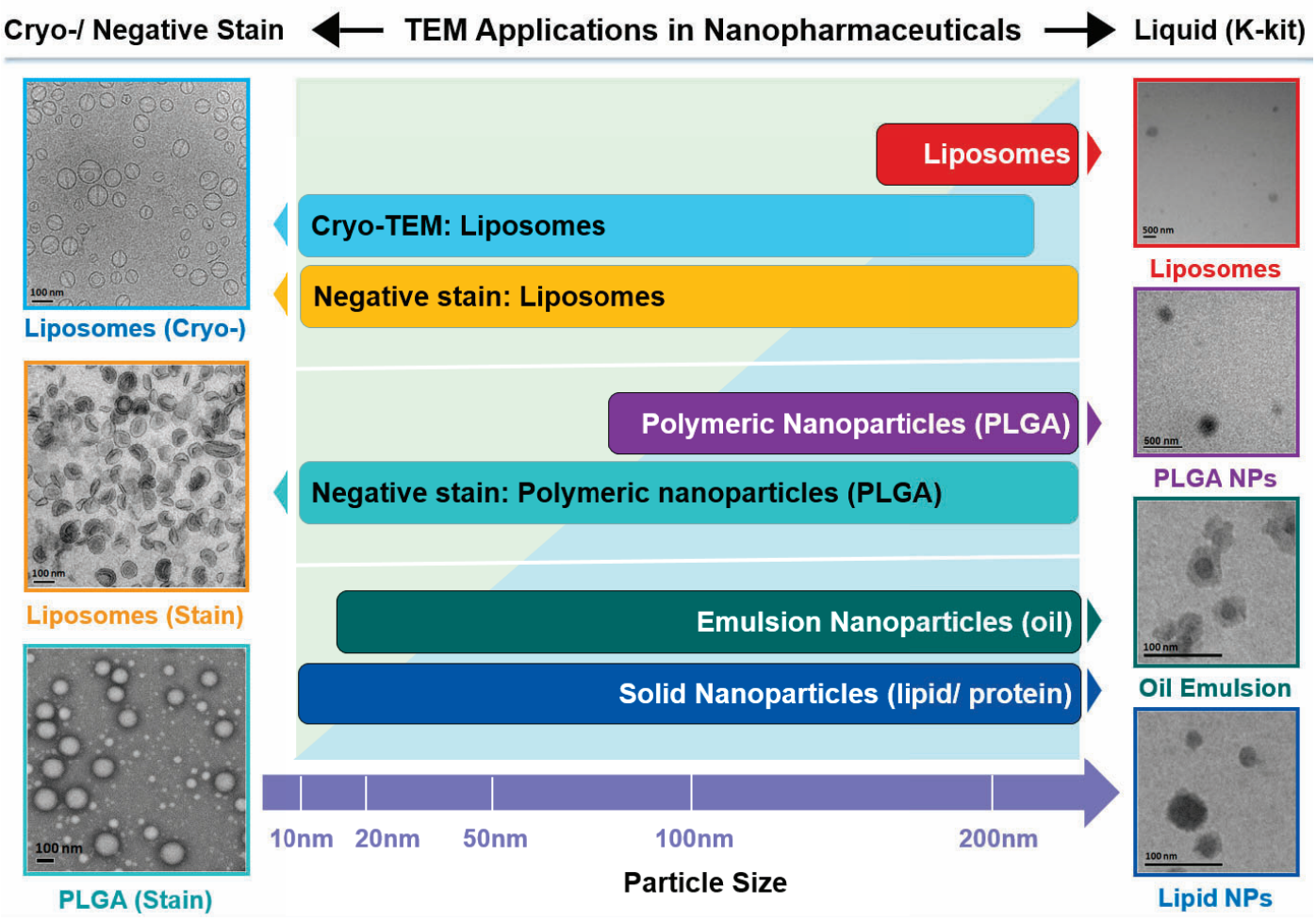
AuroVist® solution was directly loaded and sealed in a K-kit in liquid form.



Oil emulsion in water was loaded and sealed in a K-kit in liquid form.



Brand Name of Pharmaceuticals	**Doxil ® (1995 approved)**	**Abraxane ® (2005 approved)**	**Aurimune ® (Phase II)**	**Resovist ®**	**Rexin-G ® (Phase II)**
Particle Size	80-100 nm	~ 130 nm	~ 27 nm (AuNPs core), ~ 30-40 nm as hydrated	~ 45-60 nm (Hydradynamic diameter)	~ 100 nm
Particle Concentrations	1.0 x 10^{14} liposome /ml	4.3 x 10^{13} albumin particles /ml	≦ 1.7 x 10^{12} gold particles /ml	1 x 10^{14} particles /ml	1-4 x10^{11} cfu



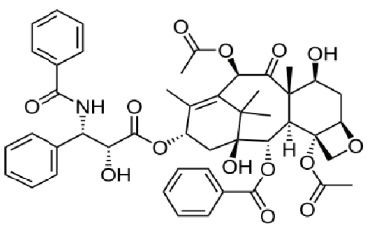
Protein Particles in Nanopharmaceuticals

K-kit can be used for characterizing protein particles in Nanopharmaceuticals by imaging the particle morphology, size and size distribution, to evaluate drug formulation or conduct any bioequivalence study.

Protein particles (Paclitaxel @ Albumin) in Abraxane®

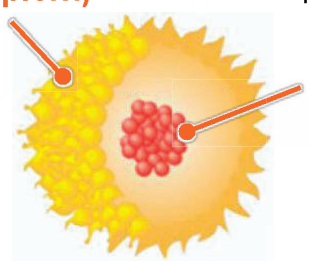
Abraxis BioScience

* US FDA approved 2005

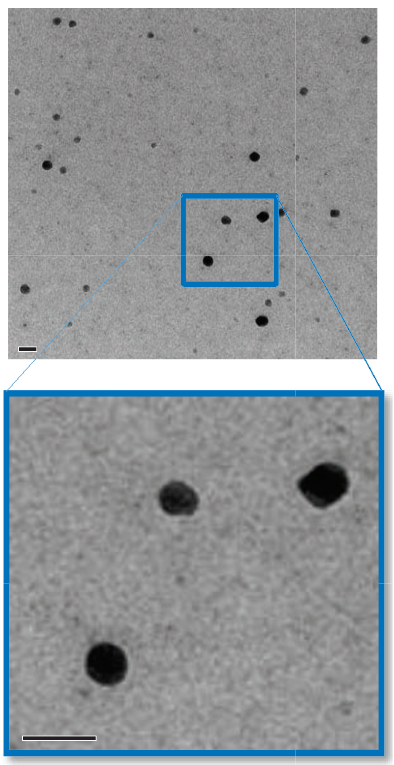


Albumin Shell (Excipient)

- Hydrophobic
- M.W. 854 Da

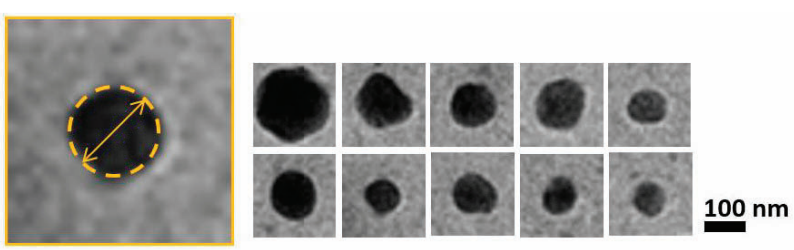


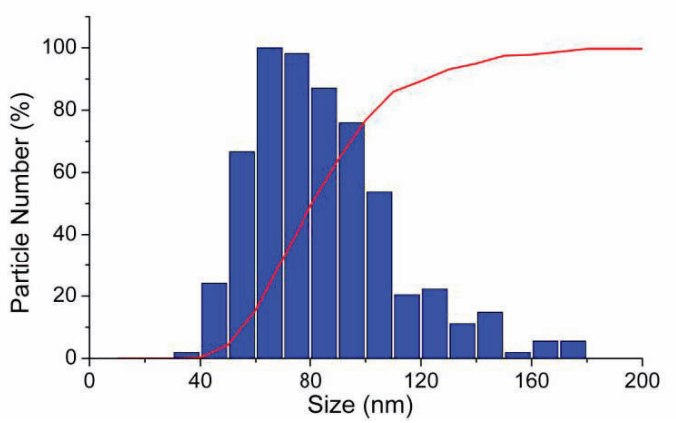
Paclitaxel (Drug)



* Scale bar: 200 nm

Abraxane in saline _ size & size distribution (D10/ D50/ D90)





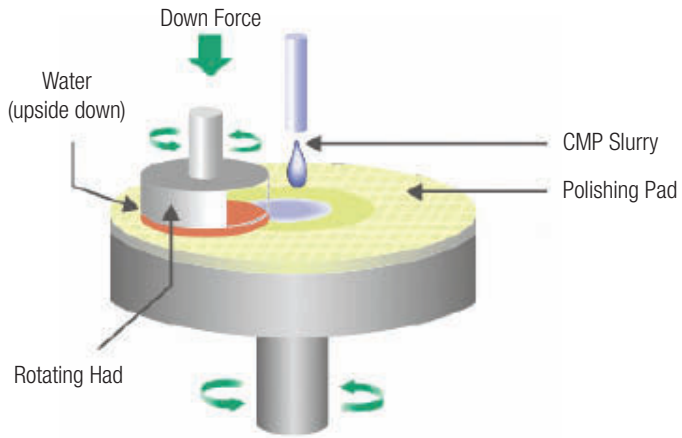
- Total calculated particle #: 319
- Average size: 85.1 nm
- Standard deviation: 27.0 nm

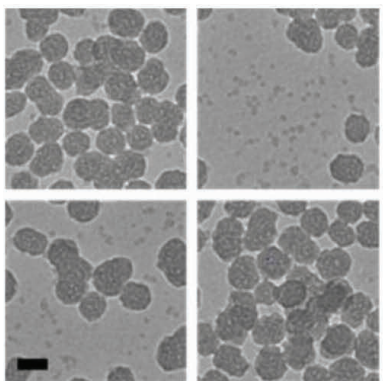
Parameter	Size (nm)
D10	55.6
D50	80.1
D90	122.2
Span: (D90 - D10) / D50	0.831

Abrasives in CMP Slurry

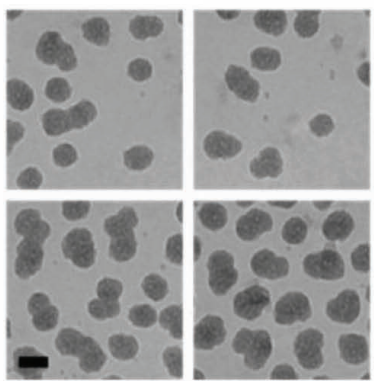
Primary and secondary particles in undiluted slurry

- **Composition**
- **Shape**
- **Size/shape distribution**
- **Aggregation state**
- **Surface**

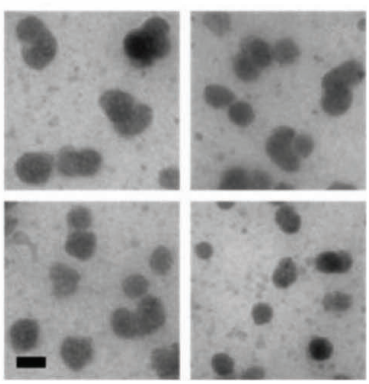




Dried on copper grid

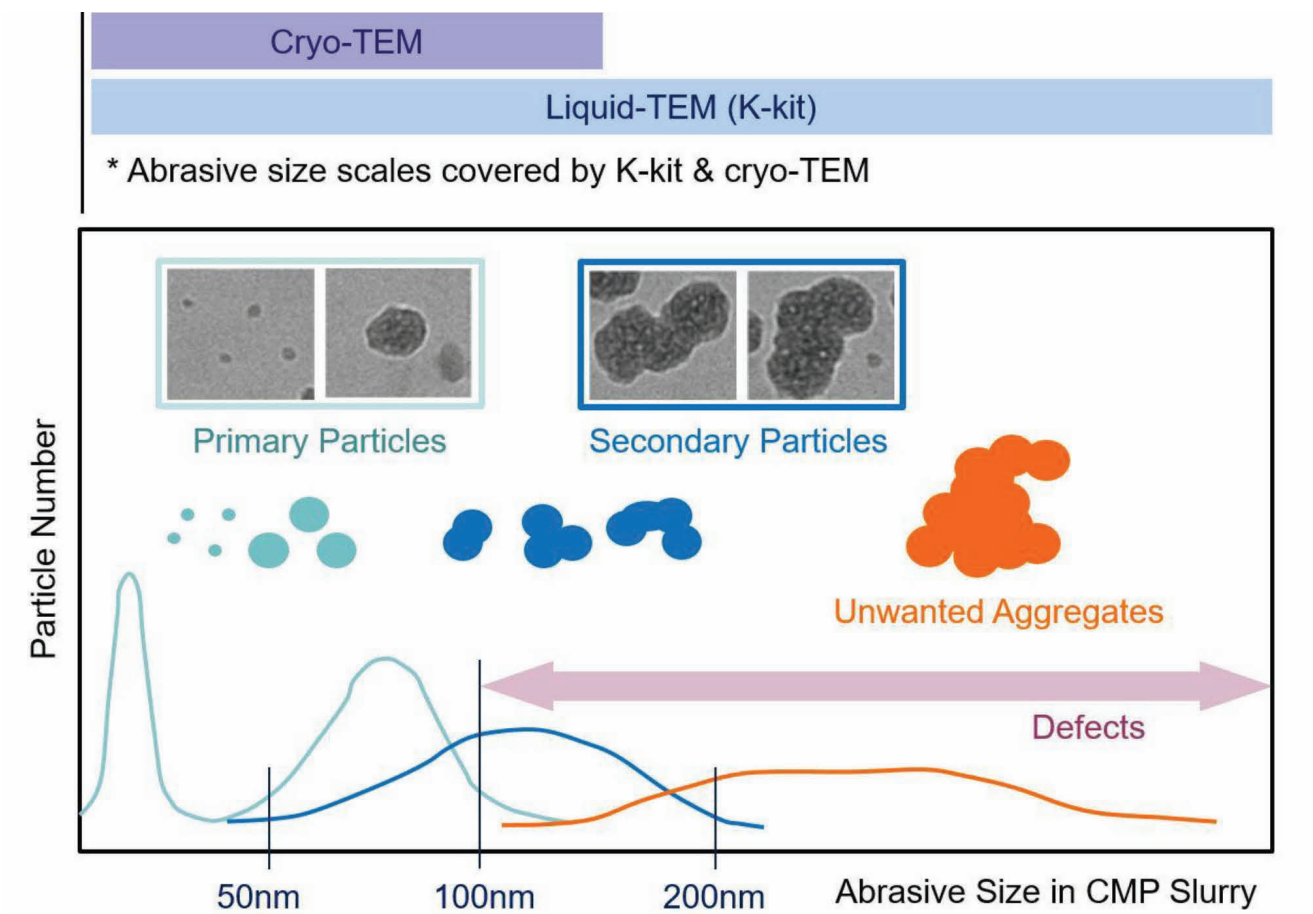


Frozen in Cryo-TEM grid



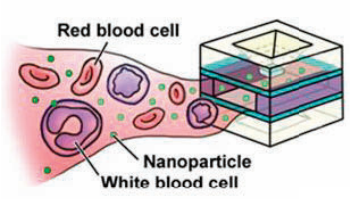
In liquid phase in K-Kit

* scale bar is 50nm



NOAAs of Au Nanoparticles (NPs) in Blood

K-kit can be used to perform in-vitro and in-vivo physicochemical characterizations of NPs in blood by TEM.



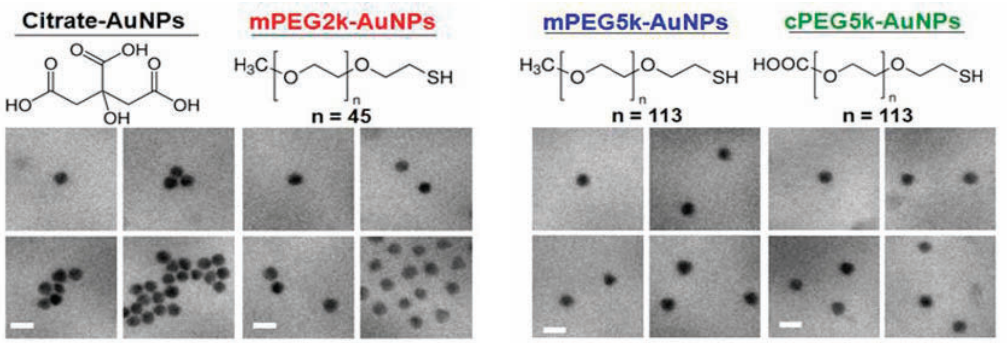
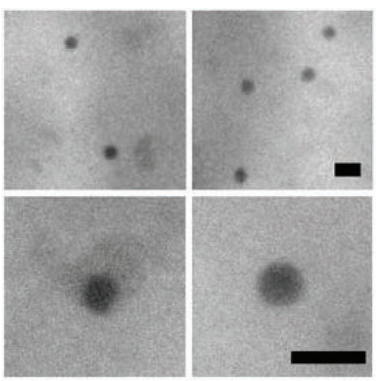


Image-based statistic analysis of particle concentration (K-kit vs. ICP-MS)



* Scale bar: 20nm

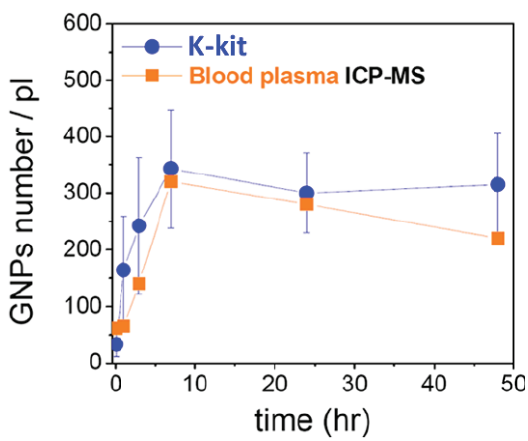
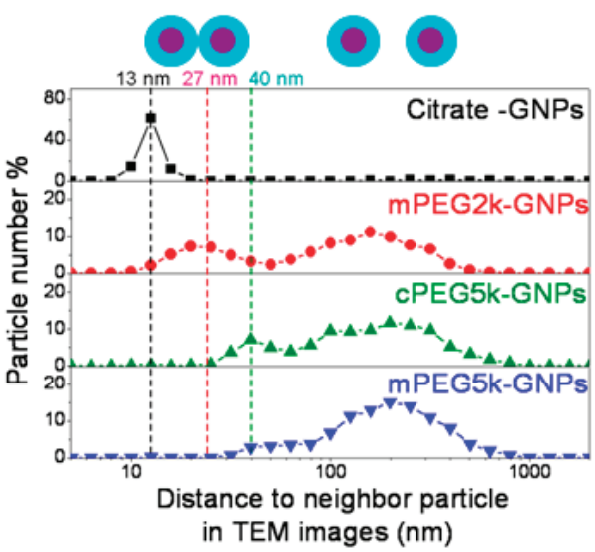
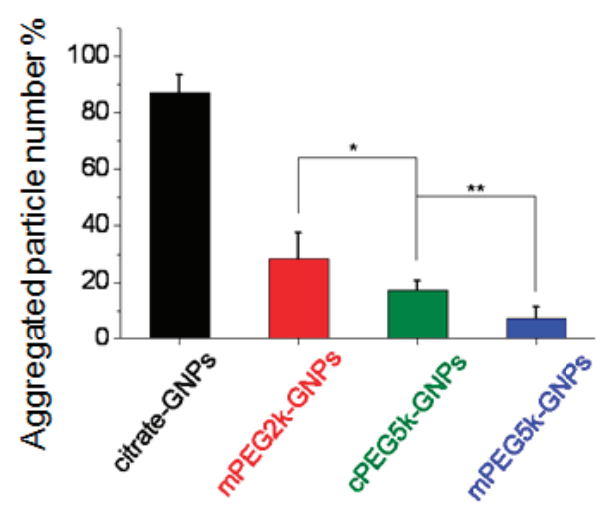


Image-based statistic analysis of aggregation and agglomeration of Au NPs in blood



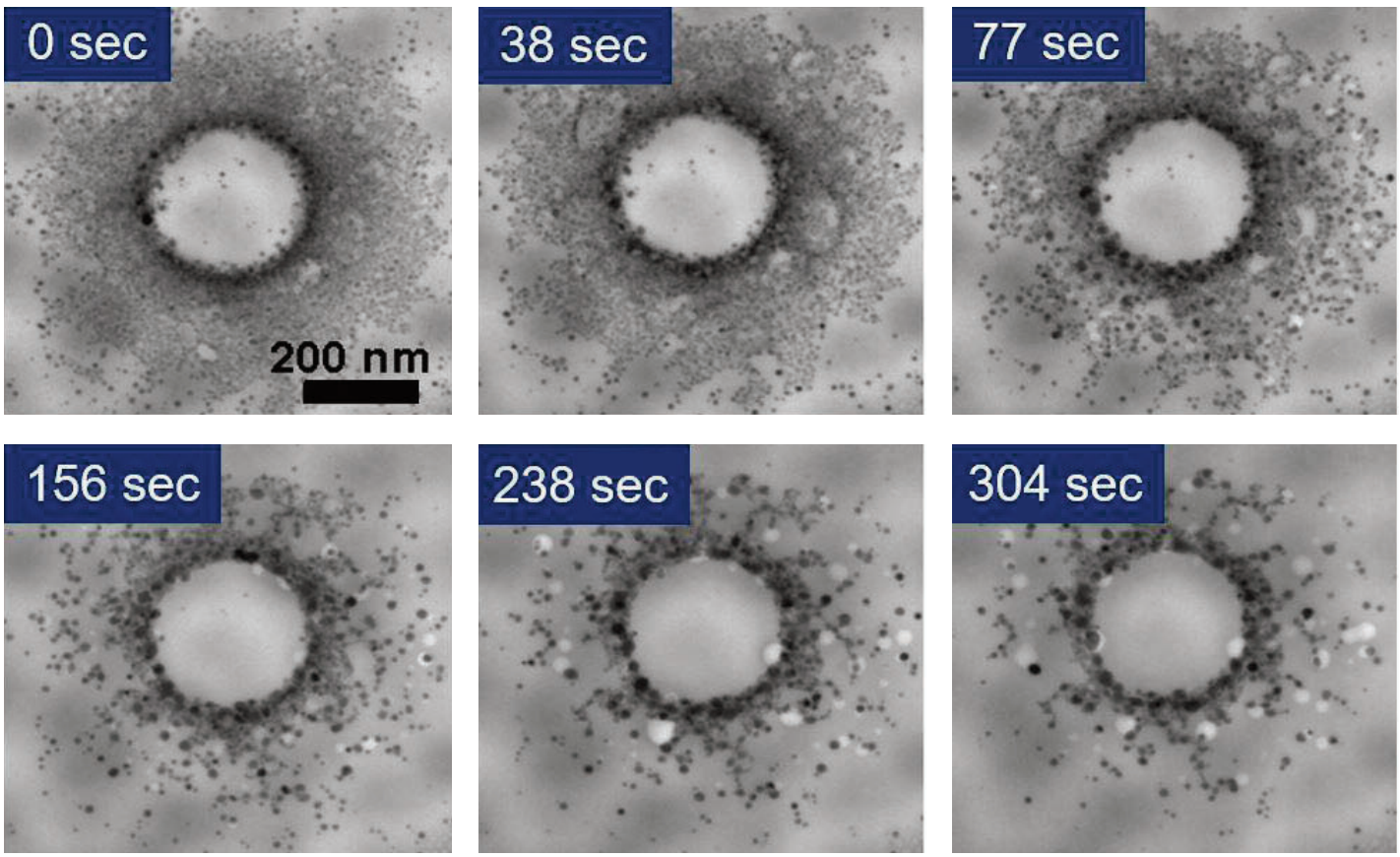


In-situ Dynamic Observation

The dynamic changes of reaction processes in liquid can be observed and studied by K-kit.

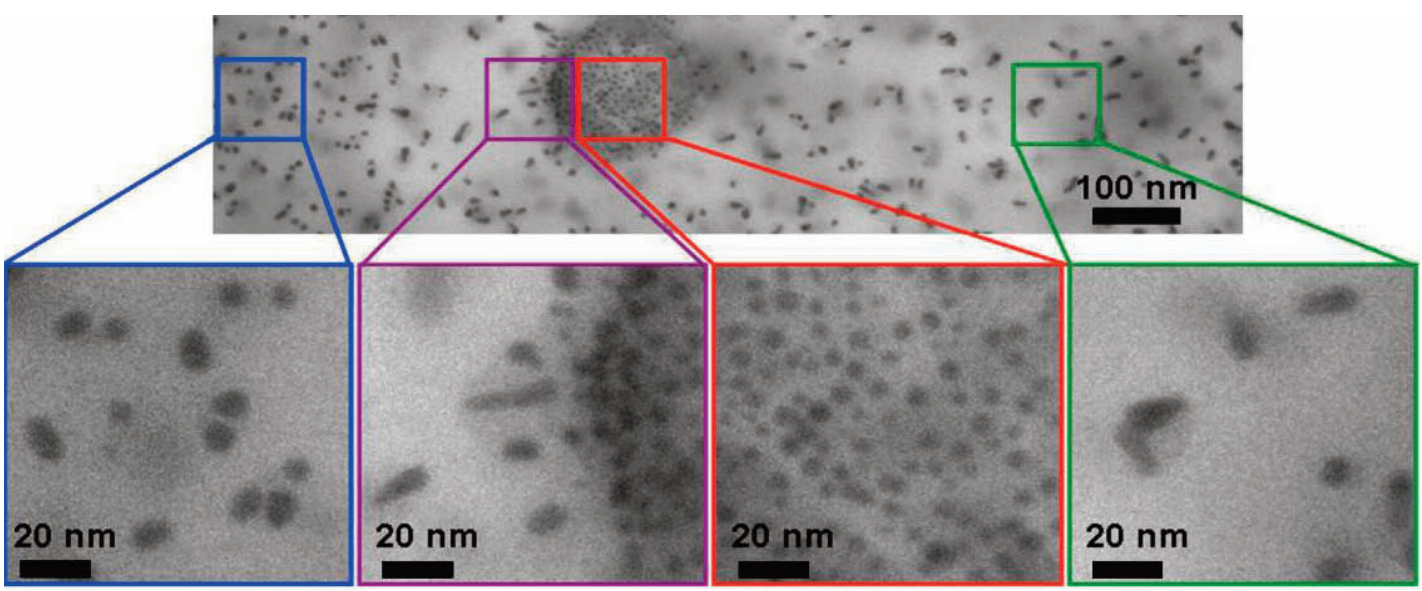
Dynamic observation of polystyrene beads in PBS buffer (sodium ion)

The reduction process of sodium ions, induced from the TEM electron energy, in PBS buffer around a polystyrene bead, could be observed.



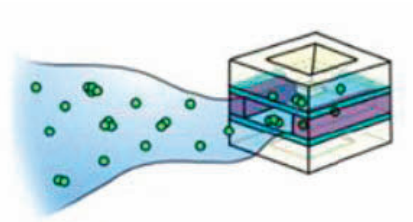
Gold metal growth in water with and without polystyrene beads

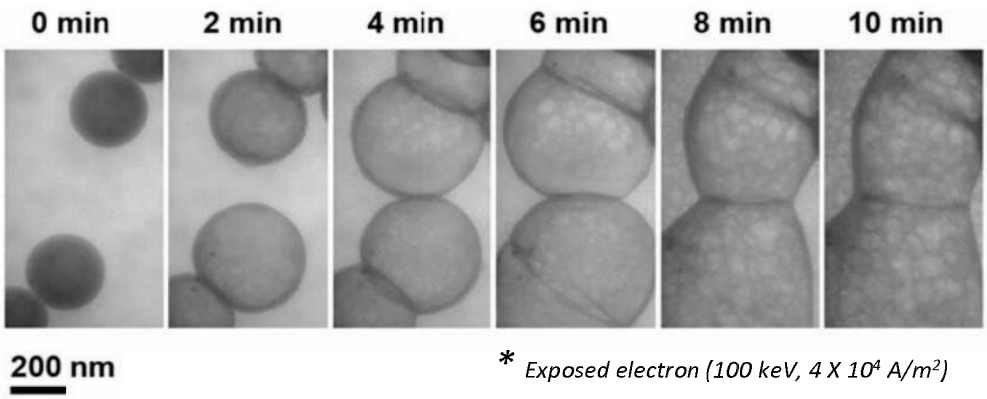
The metal growth of Au ions in water could be observed dynamically at the same time in the areas far away and nearby a polystyrene bead. (As shown in the image, there's a polystyrene bead at the center, with a lot of relatively small Au particles surrounded)



Dynamic observation of NOAAs in liquid

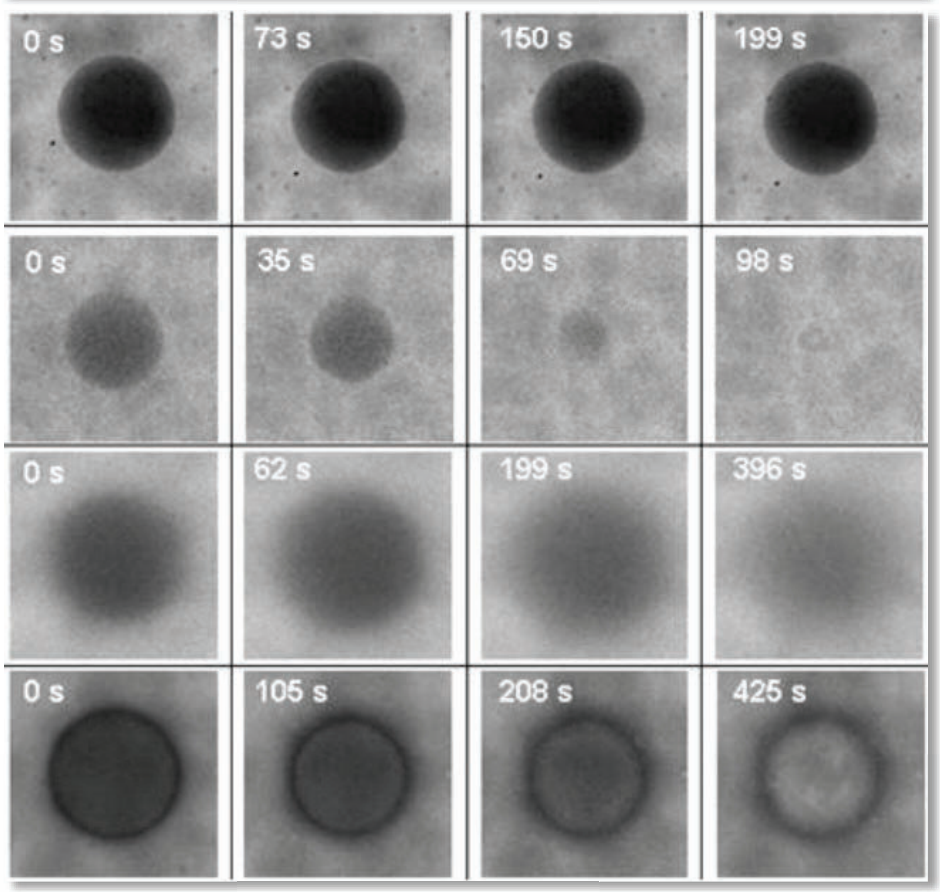
Dynamic observation of silicate nanoparticles in water





* *Exposed electron (100 keV, 4 X 10^4 A/m^2)*

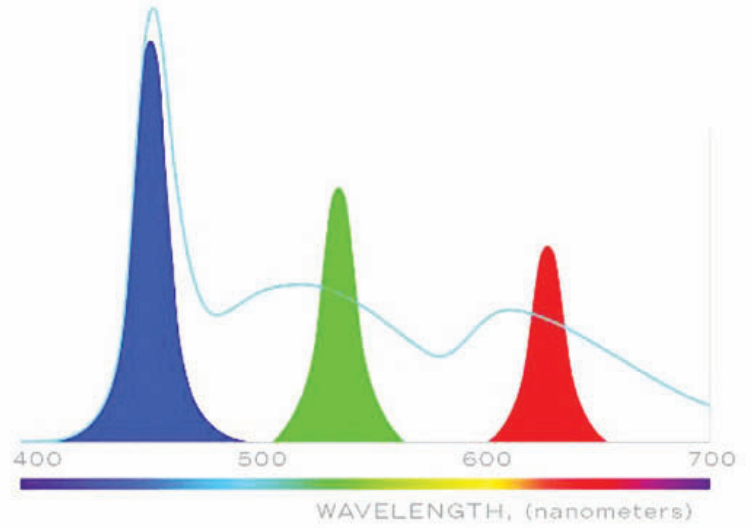
Dynamic observation of polystyrene beads

In situ dynamic observation by TEM (Hitachi H-7650)	Observation Environment
	Vacuum, 4.0 X 10^4 A/m^2
	Air, 2.5 X 10^3 A/m^2
	Water, 2.5 X 10^3 A/m^2
	Buffer/ PBS (Sodium ion), 1.0 X 10^4 A/m^2

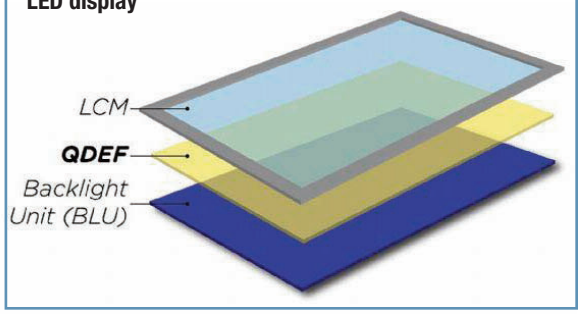
Quantum Dots in Solution

Quantum dots will enable a market for devices and components worth over $11bn by 2026.

QD: Quantum dots
BLU: Backlight Unit
LCM: Liquid Crystal Module
QDEF: Quantum-Dot Enhancement Film

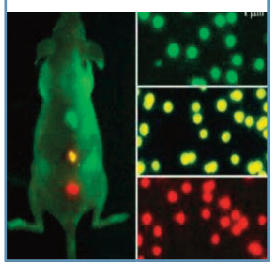


LED display

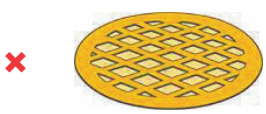


QD inks for printed electronics

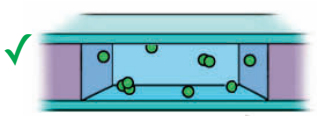
QD imaging diagnosis



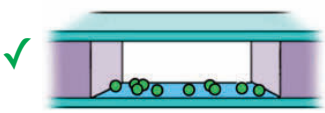
QDs dried on copper grid

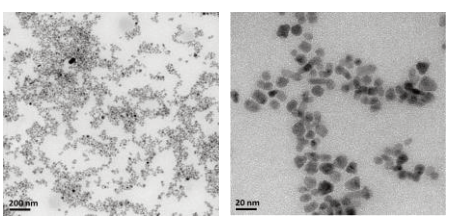


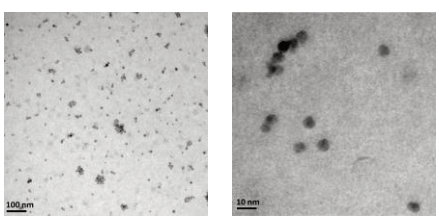
Wet mode of K-kit

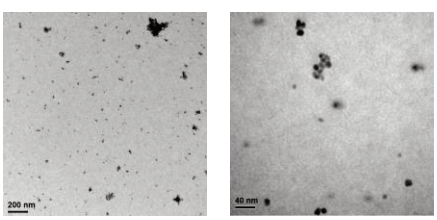


Thin Layer mode of K-kit

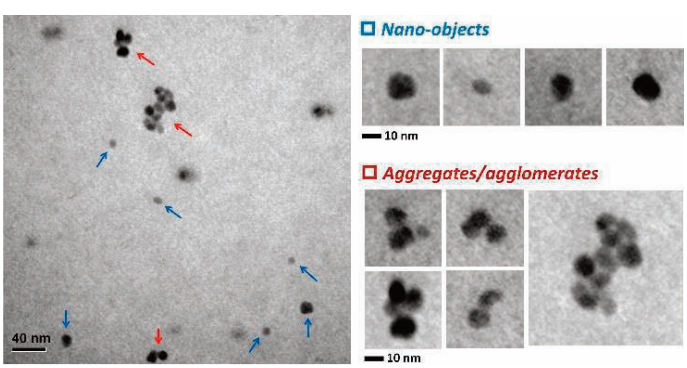


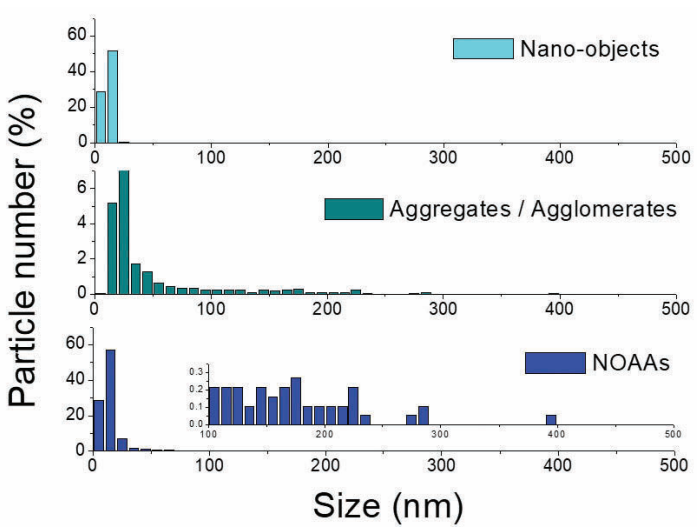






Size and Size Distribution of QDs in Chloroform (Thin Layer Mode of K-kit)

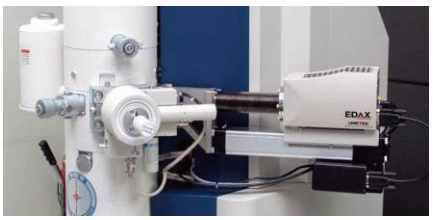


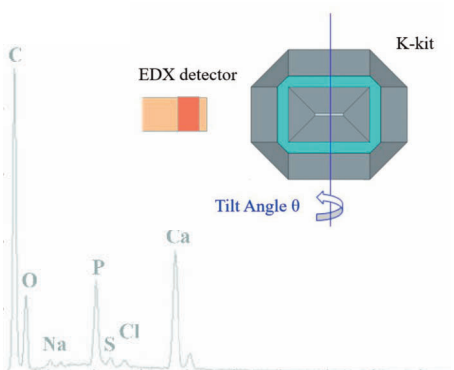


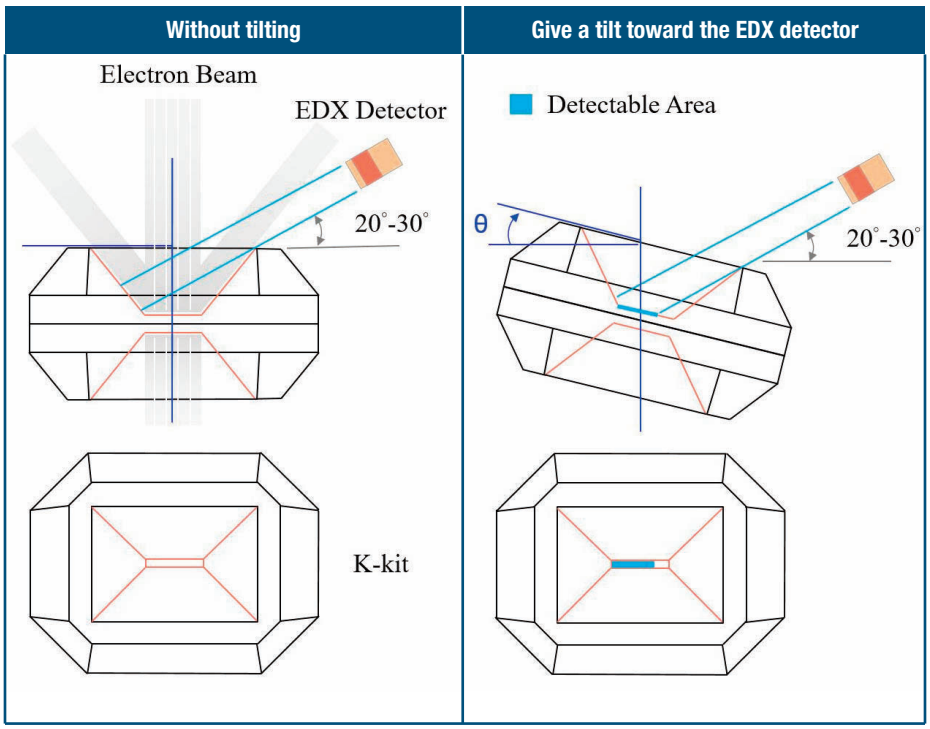
- Sample solution was directly loaded into K-kit
- Nano-objects = Primary particle
- Aggregates/agglomerates = Secondary particle

How to Make EDX Analysis Achievable on a K-kit

By pointing the window long side to the detector and tilting the holder at some angles, which could make the EDX analysis achievable on a K-kit.





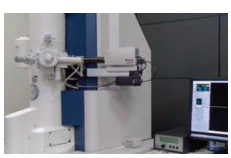


EDX available angles for different kinds of TEM equipment

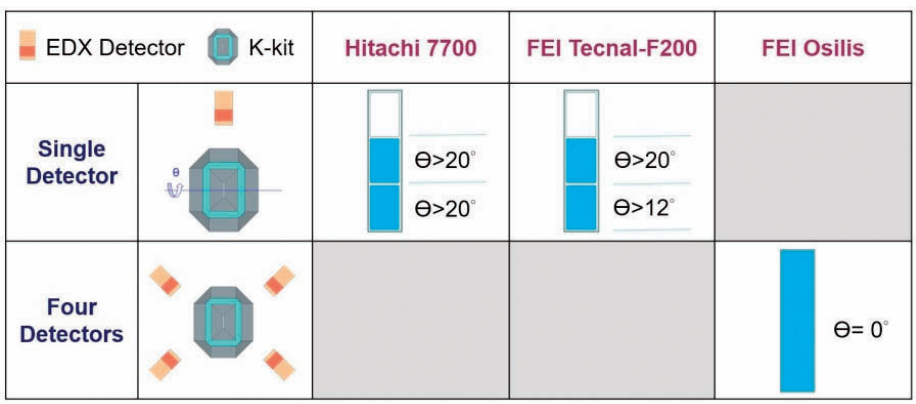
Some types of TEM installed with multiple EDX detectors usually can get a clear X-ray excited signal from K-kit, no need to turn any of body rotation or tilting.

Example

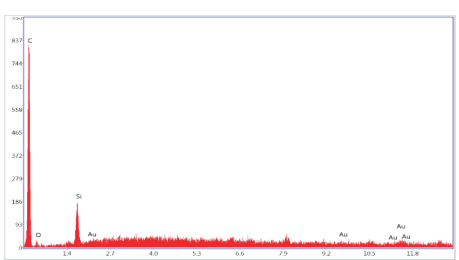
TEM: Hitachi 7700
EDX: Single Detector
Liquid Sample: $AuCl_3$
Tilt Angle: 0°, 10°, 20°



Detectable Area



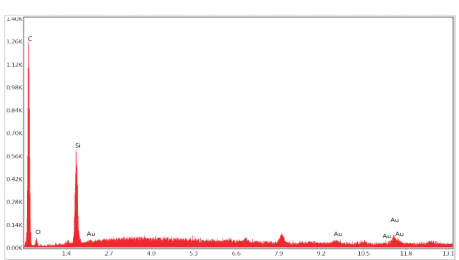
θ = 0°



Element	Weight %	Atomic %	Xpt	KAB Factor	Net Int.	Net Error %
C K	75.12	93.74	0.00	1.11	97.83	1.55
O K	0.00	0.00	0.00	1.11	0.00	100.00
Si K	9.55	5.10	0.00	1.00	13.79	8.70
Au L	15.33	1.17	0.00	7.74	2.86	53.67
	100.00					

NG

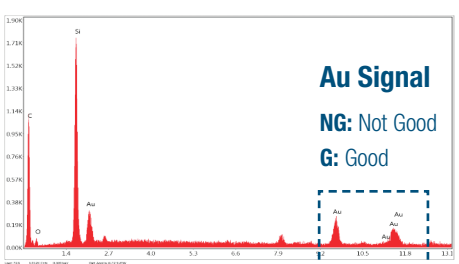
θ = 10°



Element	Weight %	Atomic %	Xpt	KAB Factor	Net Int.	Net Error %
C K	60.70	84.82	0.00	1.11	96.91	1.29
O K	0.00	0.00	0.00	1.11	0.00	100.00
Si K	23.09	13.80	0.00	1.00	40.88	3.11
Au L	16.21	1.38	0.00	7.74	3.71	31.09
	100.00					

NG

θ = 20°



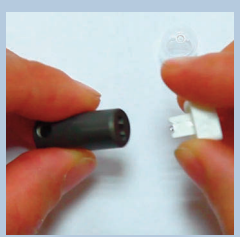
Element	Weight %	Atomic %	Xpt	KAB Factor	Net Int.	Net Error %
C K	15.85	50.58	0.00	1.11	101.26	1.36
O K	0.04	0.10	0.00	1.11	0.27	74.21
Si K	28.18	38.44	0.00	1.00	199.57	1.22
Au L	55.93	10.88	0.00	7.74	51.16	8.91
	100.00					

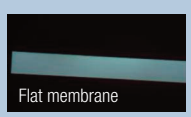
G

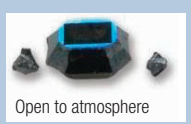
Matters needing attention when the K-kit is in use

Inspection Before Use
With Newton's rings on the membrane. (Be sure to keep the channel vacuum sealed)

Silicon body of K-kit should be damage-free.

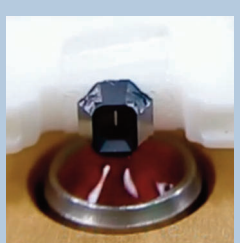


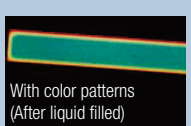


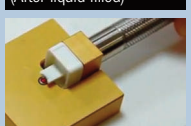


Channel Tips Removal
Be sure to remove both the channel tips before using K-kit.

It should be finished the liquid loading within 0.5 hour, after breaking the channel tips.

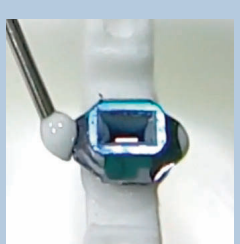


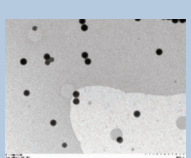




Liquid Loading
Keep the K-kit steadily touching on liquid for around 1 minute, to allow the filling to complete.

Do not immerse the K-kit in liquid.





Liquid well reserved
(glue the openings soon)

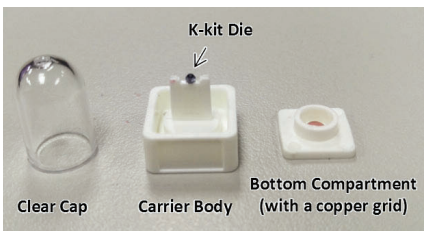
Gluing Process
Glue both ends of the channel within 1 minute after liquid loading.

Be sure not to do the channel gluing step, if making for Thin Layer mode of K-kit.

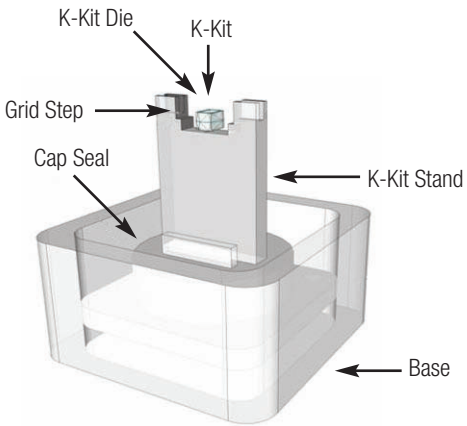
Take care during the gluing step, to avoid the glue flowing into the observation window.

SPECIFICATIONS

K-Kit Carrier



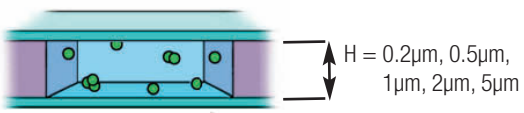
Each carrier has a K-kit attached on top, protected with a clear cap. (A copper grid is enclosed at the bottom of the carrier.)

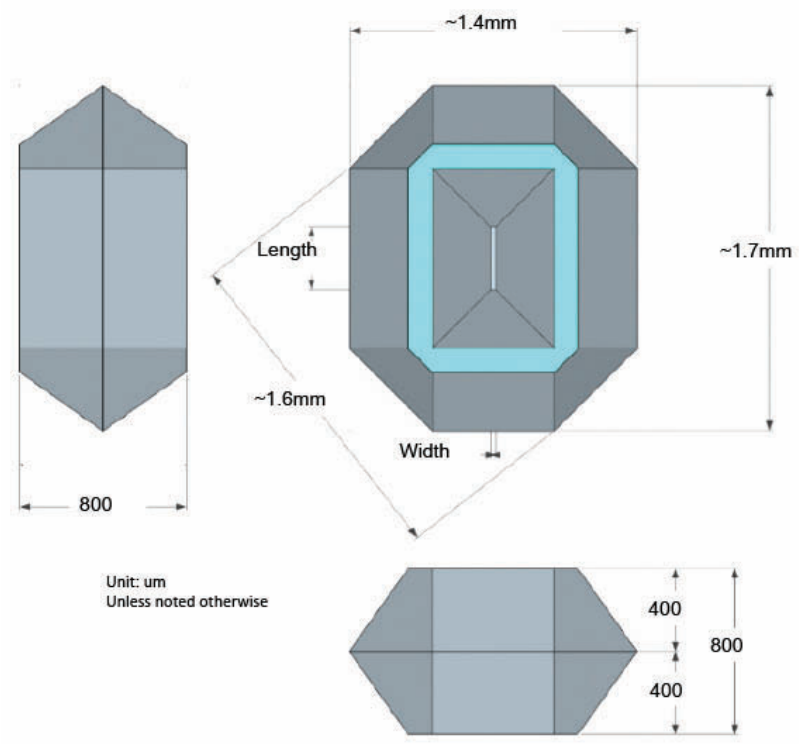


Dimensions

Window Length: 300μm, Width 25μm

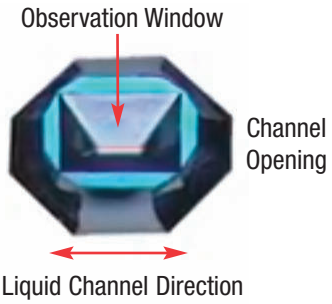
Channel Height (H): **0.2 and 2.0 standard**
0.5, 1.0 and 5.0 available

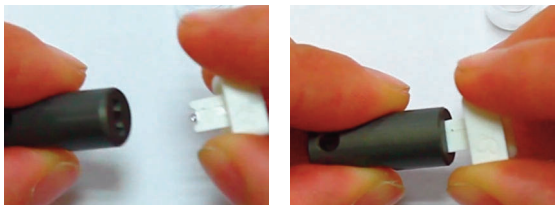




1. K-kit

K-kits are Si-based microchannel devices with silicon nitride windows that allow TEM observation. The seemingly irregular shape is a result of KOH anisotropic wet etching, which is also responsible for forming the rectangular observation window in the middle of the device. The liquid channel is parallel to the window, with openings at both ends.





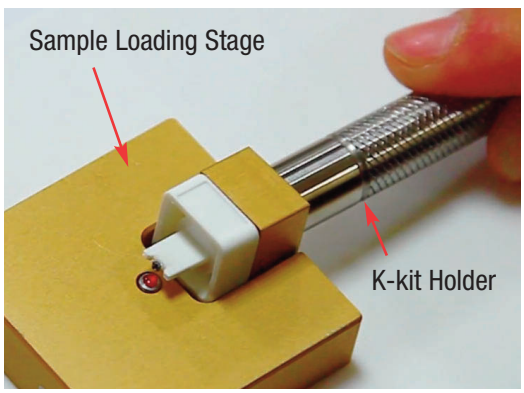
There are channel tips at each end of the channel to protect the surface condition until before use. Use the channel opener to open the channel by inserting the K-kit carrier top into the opener. Gently push in to the end. The channel opener has a self-guiding slot and a mechanism to break off the tips before the carrier top plate reaches the end.

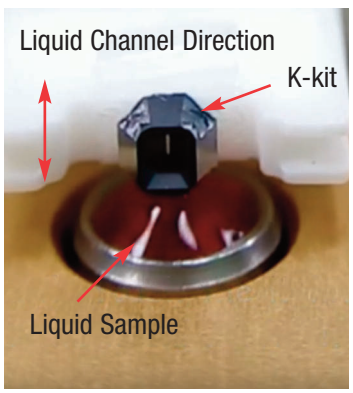
2. Liquid Loading

Place about 2 micro-liter liquid sample at the center of Sample Loading Stage. Place the K-kit carrier at the end of the K-kit holder.

Fit the notch of the holder onto the horizontal rod on the Loading Stage, forming a lever hinged on the rod. This will place the K-kit on the carrier right above the liquid drop. Lower the K-kit to make contact with liquid by gently lifting the back of the K-kit Holder.

Liquid fills the channel through capillary force. The liquid surface is "pulled up" by the K-kit. Keep the K-kit steady for approximately 1 min to allow for the filling to complete. The aqueous liquid sample should be placed on a glass slide. Both the K-kit and glass surface are hygroscopic. Do not immerse the K-kit in liquid.





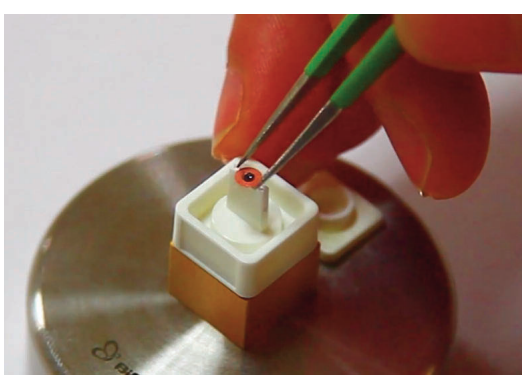
3. Vacuum Seal

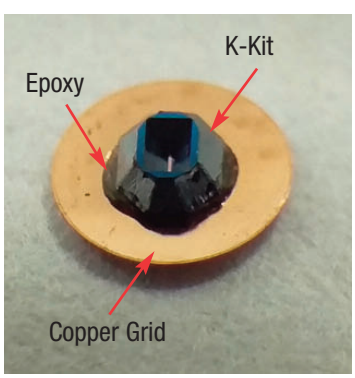
Place the K-kit carrier on the Gluing Stand. Use Needle Pen to pick and apply the seal epoxy on to the channel openings. Cover the channel openings at both ends with adequate amount of seal epoxy.

To ensure the liquid can be well reserved in K-kit, it's strongly recommended to complete the channel-sealed gluing within 1min after liquid loading.

4. Copper Grid

Keep the K-kit carrier on the Gluing Stand. Use Needle Pen to pick and apply the Mounting Glue epoxy on to K-kit peripheral. Then, place the supplied copper grid over the K-kit. The steps on carrier top plate facilitate centering and leveling the copper grid.



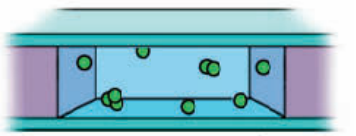


COMPONENTS

- **Tools are optional available in a Tool Set or ordered individually. The glues are also available.**
- **Figures are for illustration purposes. The tools you order may be different in color and/or from minor design changes.**

K-kits

Two gap heights (H) available: 0.2µm or 2µm. Two package options: 4 or 6 K-kits per pack. Additional gap heights and pack sizes available upon request.

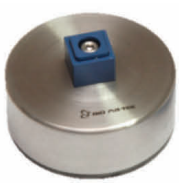


Cat. No.	Description	Qty.
K7260-402	K-kit, 0.2µm gap height	4/pk
K7260-420	K-kit 2.0µm gap height	4/pk
K7260-602	K-kit 0.2µm gap height	6/pk
K7260-620	K-kit 2.0µm gap height	6/pk

K-kit Tool Box

The K-kit Tool box houses a full tool set, including K-kit holder, Sample Loading Stage, Needle Pen, Gluing Stand, Channel Opener, Sealing Glue, Mounting Glue, Glass Slides, 6/pk of K-kits (2.0µm or 0.2µm gap height), Shipping Box (empty), and some replacement parts.

Cat. No.	Description	Qty.
K7261-R	K-kit Tool Box, 2.0 µm, Silver, includes full tool set	each
K7261-S	K-kit Tool Box, 0.2 µm, Silver, includes full tool set	each

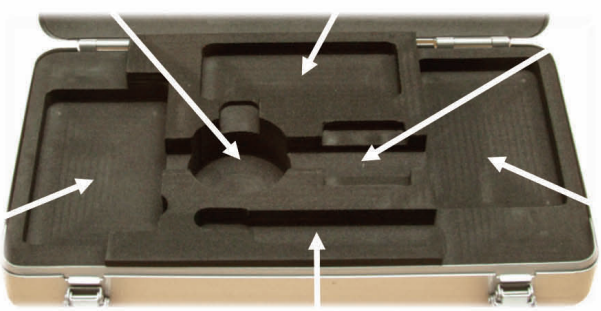


K-kit Gluing Stand

Glass-slide Pack
(Six slides inside for free)

Sample-loading Stage

Accessory Box



K-kit Holder & Needle Pen

K-kit Shipping Package
(Without K-kits)

ACCESSORIES

K-kit Holder

The K-kit Holder consists of an anodized aluminum header and a stainless steel handle. The K-kit carrier fits on the header (after removing the bottom compartment). When the notch on the side of the header fits over the horizontal bar on the Loading Stage (see below), the K-kit on the carrier attached on the header will be just above the liquid sample.

Cat. No.	Description	Qty.
K7263	K-kit Holder	each

Needle Pen

The Needle Pen is designed to facilitate the K-kit gluing operation. It has a thin needle 3.0 mm long and 0.27 mm in diameter. The thin needle makes it convenient to pick just enough glue (of the order of 0.1µl[ix]) for sealing the channel openings and (around 1µl[ix]) for mounting the copper grid. The needle is made of stainless steel. It is strong, yet slightly flexible, suitable for the job.

Notes:

It is important to keep the needle free of residue glue. Please wipe the needle clean right after each use. It will be practically impossible to clean the needle once residue glue on it cures.

The needle is held in place in the pen by a set screw on the side of the pen. A replacement needle and a small Allen key are provided with each Needle Pen. The needle is sharp. Please handle with care.

Cat. No.	Description	Qty.
K7265	Needle Pen	each

Sample-Loading Stage

The Loading Stage consists of an anodized aluminum body. It has a horizontal bar in a recess on the side and a hole in the middle to house the Liquid Stage, which is a removable stainless steel rod. The removable design is for easy cleaning. The horizontal bar defines the rotational axis for the K-kit Holder, which has a notch on the header to fit on the horizontal bar.

Cat. No.	Description	Qty.
K7264	Sample Loading Stage	each

Gluing Stand

The Gluing Stand has a stainless steel base and an anodized aluminum header, which is much like the header on the K-kit holder, without the notch on the side. The Gluing Stand keeps the K-kit carrier in place for gluing work.

Cat. No.	Description	Qty.
K7266	Gluing Stand	each

Channel Opener

The Channel Opener is used to remove the channel tips, while the K-kit stays on the carrier. It's made of anodized aluminum with a cut-off slot design at one end.

Cat. No.	Description	Qty.
K7269	Channel Opener	each

Accessory Box

The Accessory Box contains sealing and mounting glues, four plastic sticks, and spare parts, including a spare needle, an Allen key for the Needle Pen, a Channel Opener, and two Liquid Stages. (The label can be redesigned.)

Cat. No.	Description	Qty.
K7267	Accessory Box	each

Starter Box

The Starter Box contains all of the essentials for K-kit loading. It consists of glues, a beaker, four stirring sticks, and two stainless steel thin needles.

Cat. No.	Description	Qty.
K7268	Starter Box	each

Glue Box

The Glue Box contains recommended sealing and mounting glues, and four plastic stirring sticks.

Cat. No.	Description	Qty.
K7272	Glue Box	each

Copper Grids

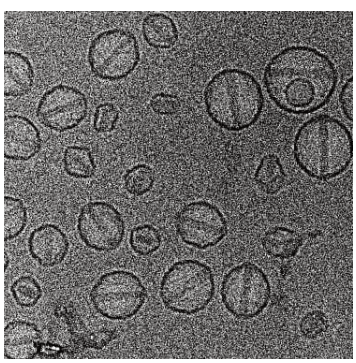
Ten pieces of Copper Grid per pack.

Cat. No.	Description	Qty.
K7270	Copper Grid	10/pk

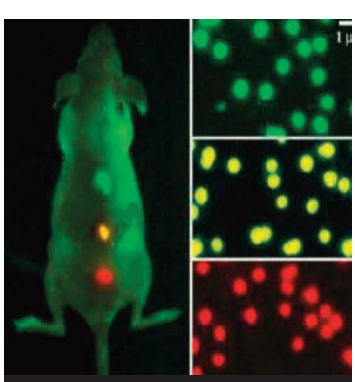
Slide-Glass Pack

Six glass slides per pack.

Cat. No.	Description	Qty.
K7271	Slide-Glass Pack	6/pk



Electron Microscopy Sciences



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