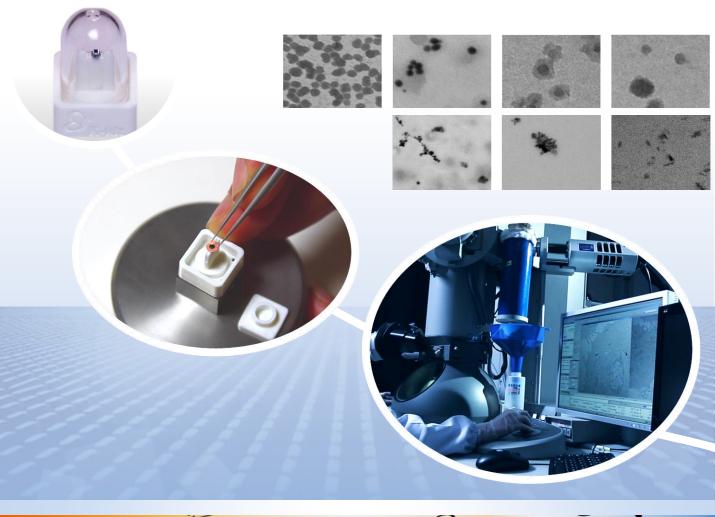
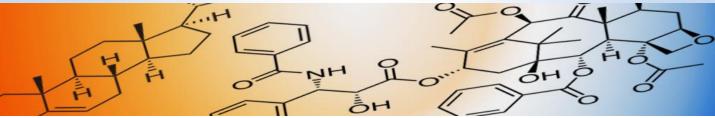


A Specimen Holder for Liquid Sample Analysis in TEM



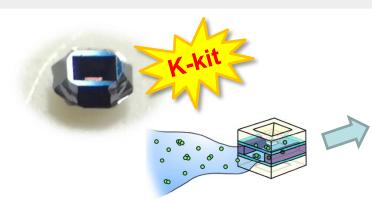


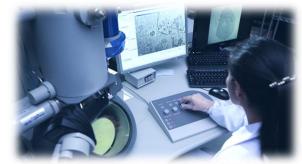




An Innovative Specimen Holder for Liquid Analysis in TEM

- K-kits are sample holders designed to be used for microscopy observation of liquid samples, allowing nanoobjects, aggregates, and agglomerates (NOAAs) in liquid samples to be imaged and characterized in TEM, FIB, and STEM etc. microscopes.
- 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.

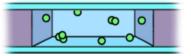




Patents being issued and publication

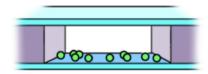
- ✓ US 7807979 B2
- ✓ US 8969827 B2
- ✓ Anal. Chem. 2012, 84: 6312-6316





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

Thin Layer

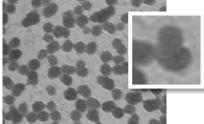


(Loaded liquid was dried out by pumping.)

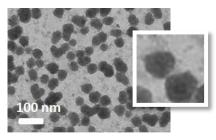
• A patented liquid drying protocol preserves the original morphology and physical state of nanomaterials with improved imaging resolution.

12 50

Transmission Electron Microscope (TEM)



(Acceptable image quality with liquid inside the K-kit.)

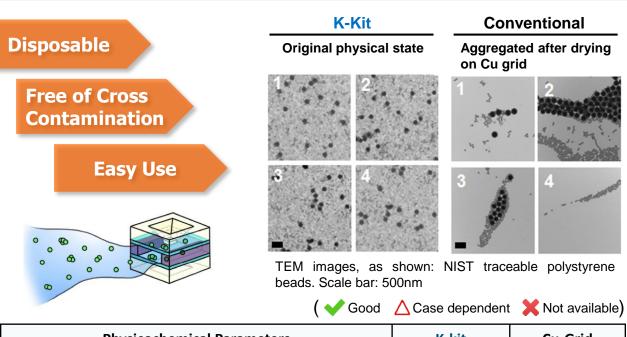


(Very good image quality, when liquid was dried out by pumping.)

TEM images shown: Undiluted Chemical-Mechanical Polishing (CMP) slurry directly loaded into K-kit.



Product Features



Physicochemical Parameters	K-kit	Cu-Grid
1. Composition	~	~
2. Size	~	✓
3. Shape	~	~
4. Size distribution	~	\bigtriangleup
5. Aggregation and agglomeration in liquid	~	×
6. Particle concentration	~	×
7. Liquid TEM observation	~	×

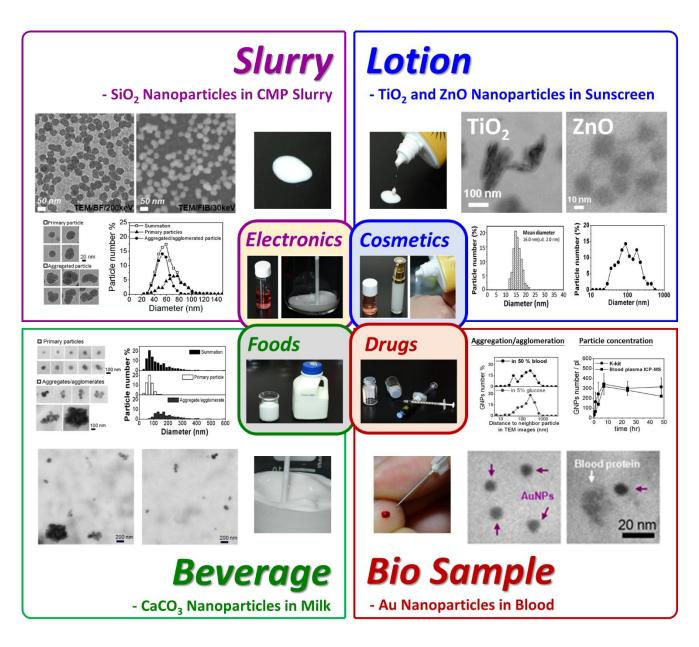
Compared with other products

Company	Bio MA-Tek	Protochips	Hummingbird Scientific	
Liquid Cell	K-kit	E-chip	TEM Holder	
Chip Size	1.4 x 1.7 mm (fits in 3 mm grids)	4 x 6 mm		
Special holder	No need	needed	needed	
Cost	US. 100-200	US. 100-200 (+Special Holder: US.100K)	US. 150K	



Applications

• Characterize NOAAs in electronics industry, cosmetics, foods, medical devices, and drugs.

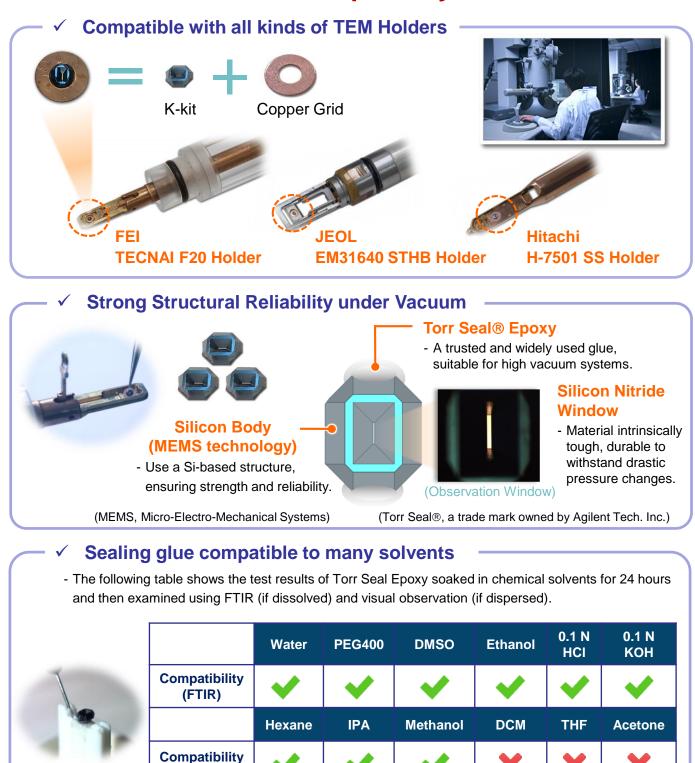


Reference :

BIO Ma-TEK

- 1. US FDA 2012, Guidance for Industry Safety of Nanomaterials in Cosmetic Products.
- 2. EU/JRC July 2012, Requirements on Measurements for the Implementation of the European Commission Definition of the Term "Nanomaterials".
- 3. ISO/TR13014: 2012, Nanotechnologies -- Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment.
- 4. ICCR 2012, Characterization of Nanomaterials II Insolubility, Biopersistence and Size Measurement in Complex Media.

K-kit Adaptability



(FTIR, Fourier Transform Infrared Spectroscopy)

BIO Ma-TEK

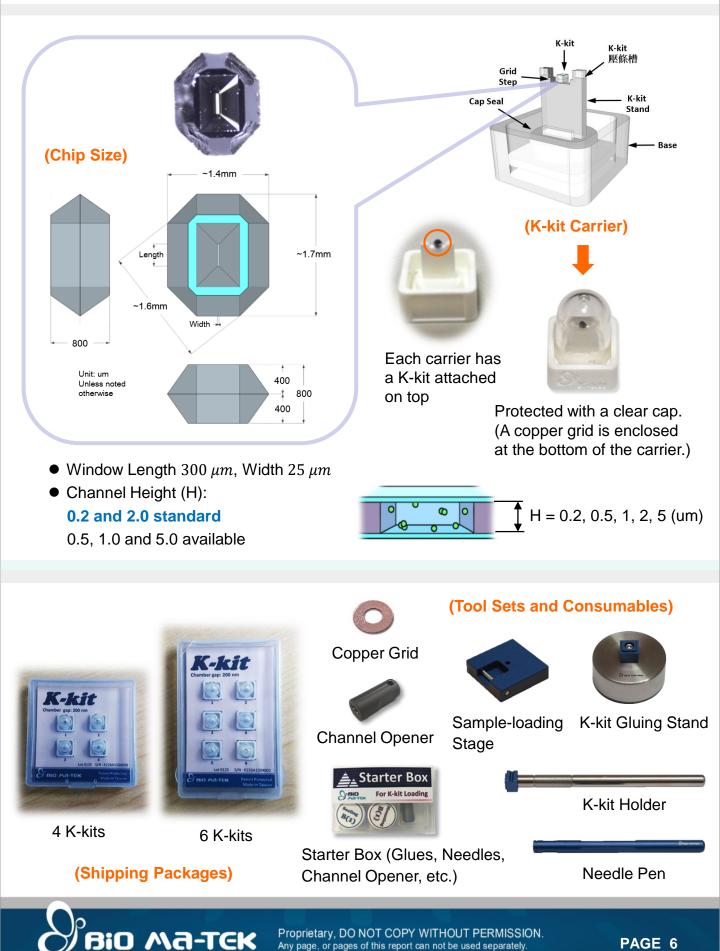
(FTIR)

Compatible (FTIR not detected)

X Use with care (FTIR detected)

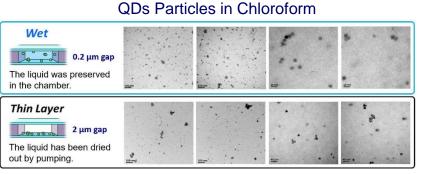
% If you would like to learn more about K-kit or have any question about its applications, do not hesitate to contact us. To enable potential customers to experience the benefits of K-kit, we offer preloaded demo samples for free. Contact us at E-mail: sales@bioma-tek.com

Shipping Packages and Tool Sets



K-kit Meets All Needs for Liquid TEM





Available with undiluted solution.

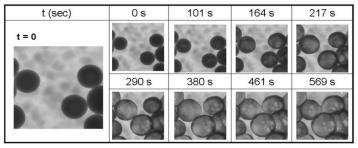
Why K-kit?

• Preserve the original morphology and physical state in liquid.

In-situ Observation

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

Dynamic Observation of Silicate Nanoparticles

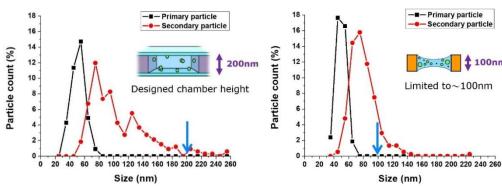




Quantitative Analysis

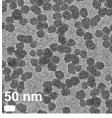
Software of image recognition for nanoparticle size distribution analysis.



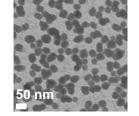


Abrasives in CMP Slurry (K-kit vs. Cyro)

Compatible to Versatile Microscopy Analyses

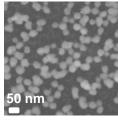


FEI-TEM @200Kev



Hitachi-TEM @100Kev

BIO Ma-TEK



FEI-STEM @30Kev

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

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100nm

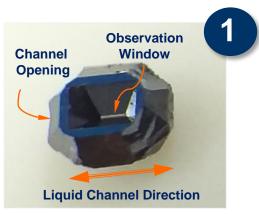
K-kit Tool Box

• Tool box, we offer a full tool set, including K-kit holder, sample-loading stage, needle pen, K-kit gluing stand, recommended glues, glass slides and some replacement parts.



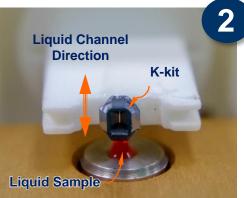
※ K-kit tool box can be in silver or vermillion colors

Sample-loading Procedure



1.K-kit:

K-kits are Si-based microchannel devices with silicon nitride windows that allow SEM, FIB, STEM, and TEM observations. The shape is a result of anisotropic wet etching. The liquid channel is parallel to the window, with openings at both sides.



2.Filling:

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 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 to prevent from the window being contaminated.





3. Torr-seal:

Cover the channel openings at both ends with Torr Seal epoxy after filling the device with liquid. (No need to do this gluing step, if one would like to dry out the liquid and leave the nanoparticles a Thin Layer mode in K-kit.)

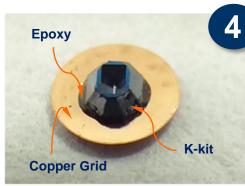
4. Copper grid:

Use epoxy to mount the sealed K-kit to a copper grid by fitting it to the precut hole at the center of the grid.



QR code link to demo video

BIO Ma-TEK





If on-line, please click the link to watch demo video: https://youtu.be/0QDvI0UTBL





Wet and Thin Layer Mode of K-kit

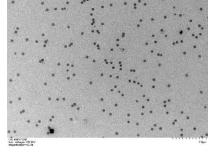
Sample Preparation	Wet Mode	Thin Layer Mode	
	With Liquid	Dried	
Inner Status of K-kit	8	00000	
Imaging Resolution	Good	Excellent	
Gap Size (Considered)	300~500nm	2000〜3000nm	
Particle Size (Loadable)	10nm \sim 300nm	3nm~2000nm	
Particle Shape	Keeping original Potentially, cou deformed.		
Chemical Reduction or Potential Damage by Electron Energy	High	Low	

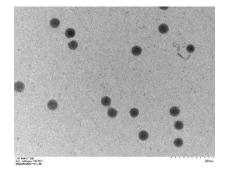
If making a Thin Layer (Dried) mode of K-kit, it's essential to keep both ends of the channel open to atmosphere, no need to do the channel gluing step.

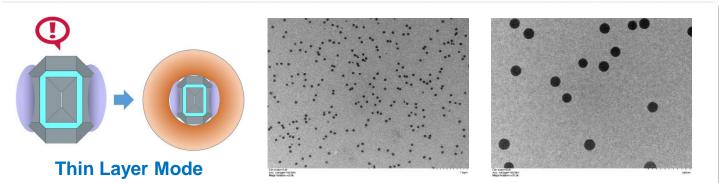
Gap Height (um)	0.1	0.2	0.5	1.0	2
Wet Mode	•	•	●		
Thin Layer Mode	Ð	•	•	●	•



вю ма-тек

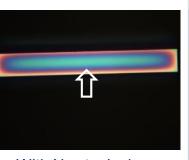






Matters Needing Attention When K-kit in Use



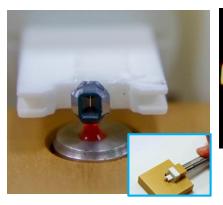


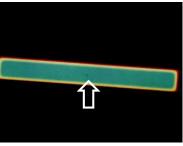
With Newton's rings (Sealed by channel tips)



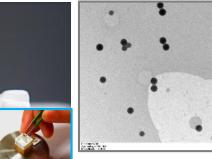


Flat membrane (Open to atmosphere)





With color patterns (After liquid filled)



Liquid well reserved (Soon to glue the openings)

Inspection Before Use

- With Newton's rings on the membrane. (Be sure the channel to keep vacuum sealed)
- Free of any damage on Silicon body of K-kit.



- 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.

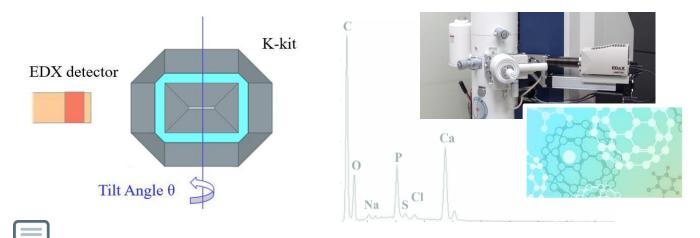
Gluing Process

- Glue both ends of the channel within 1 minute after liquid loaded.
- Be sure not to do the channel gluing step, if making for Thin Layer mode of K-kit.
- Doing the gluing step with care, to avoid the glue flowing into the observation window.

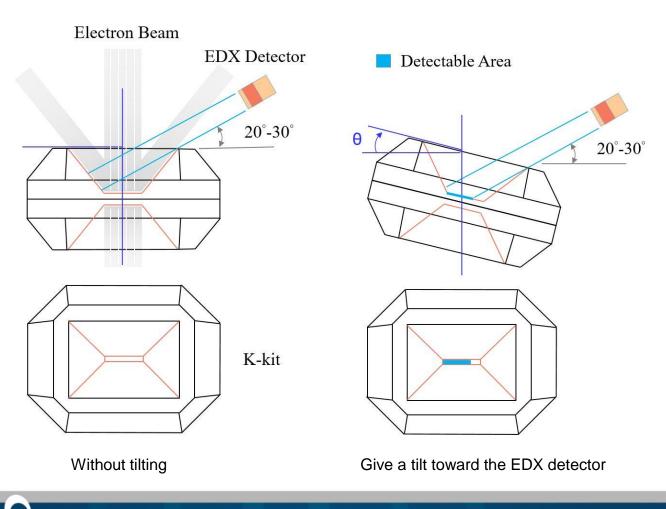


Available for EDX Analysis (1/2)

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 EDX analysis achievable on a k-kit.

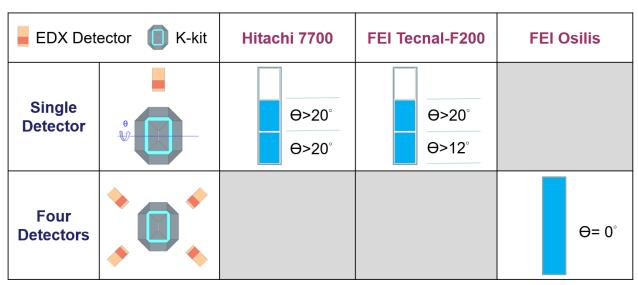




Available for EDX Analysis (2/2)

EDX available angles for different kinds of TEM equipment

Detectable Area

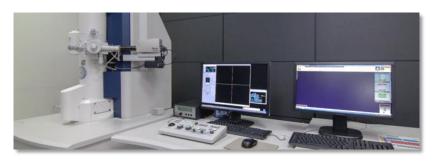


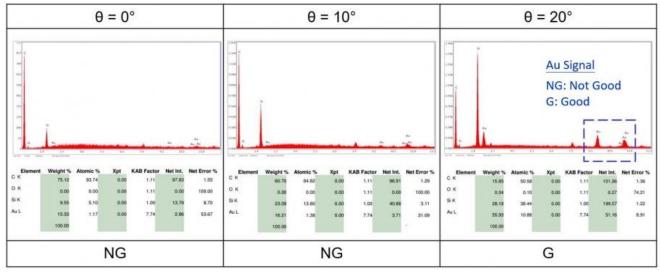
For some types of TEM installed with multiple EDX detectors, they usually can get a clear X-ray excited signal from K-kit, no need to turn any of body rotation or tilting.

Example:

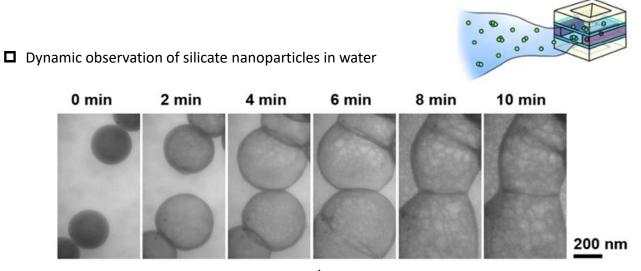
- TEM: Hitachi 7700
- EDX: Single Detector
- Liquid Sample: AuCl₃
- Tilt Angle: 0°, 10°, 20°

Bio Ma-Tek





Dynamic observation of NOAAs in liquid



* Exposed electron (100 keV, 4 X 10⁴ A/m²)

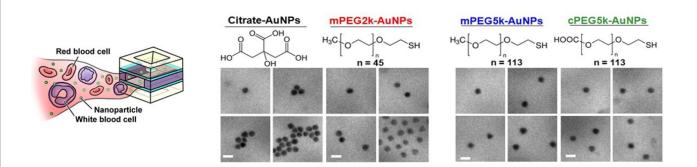
Dynamic observation of polystyrene beads

In situ dynamic observation by TEM (Hitachi H-7650)				Observation Environment
0 s	73 s	150 s	199 s	Vacuum, 4.0 X 10 ⁴ A/m ²
0 s	35 s	69 s	98 s	Air, 2.5 X 10 ³ A/m ²
0 s	62 s	199 s	396 s	Water, 2.5 X 10 ³ A/m ²
0 s	105 s	208 s	425 s	Buffer/ PBS (Sodium ion), 1.0 X 10 ⁴ A/m ²



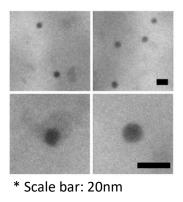
NOAAs of Au nanoparticles (NPs) in blood

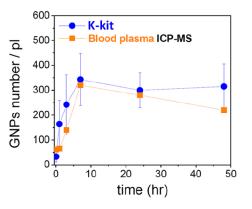
 K-kit can be used to perform in-vitro and in-vivo physicochemical characterizations of NPs in blood by TEM.



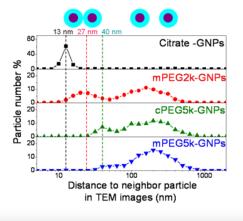
□ Image-based statistic analysis of particle concentration (K-kit vs. ICP-MS)

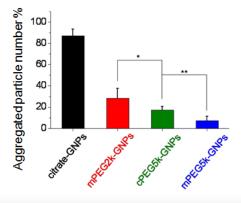






□ Image-based statistic analysis of aggregation and agglomeration of Au NPs in blood

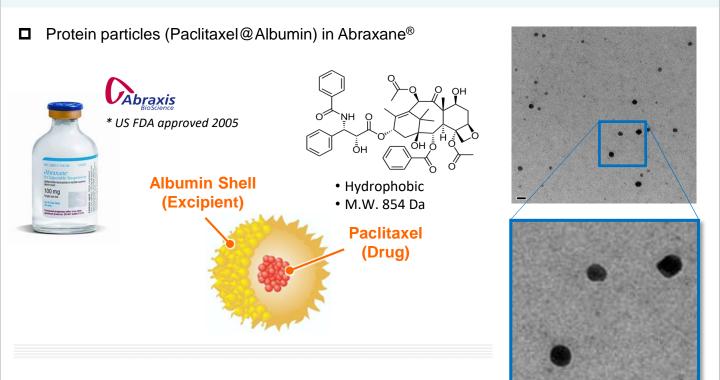






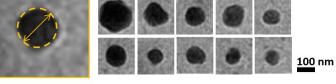
Protein particles in Nanopharmaceuticals

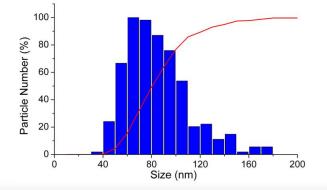
 K-kit can be used for characterizing protein particles in Nanopharmaceuticals by imaging the particle morphology, size and size distribution, to evaluate drug formulation or conduct any bioequivalence study.



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

* Scale bar: 200 nm





- 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



NOAAs of CaCO₃ NPs in milk

 K-kit can be used for characterizing nanoobjects of foods in final product form, to evaluate the safety risks of nanomaterials in food additives and in substances in contact with foods.

Comprehensive physicochemical characterization

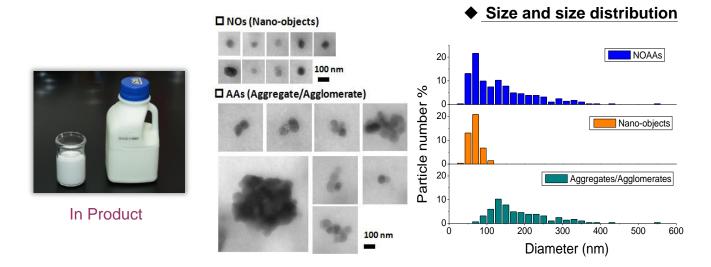
P	arameter	Results	Methods		
1	Composition	Calcite CaCO ₃	TEM/EDX, XRD		
2	Size / size distribution	Average Diameter / Standard deviation			
	Crystal particle size	36 / 4 nm	XRD		
	Primary particle size	73 / 26 nm	TEM		
	Powder size	17 / 10 μm	SEM		
3	Shape	Cubic	TEM		
4	Aggregation/Agglomeration				
	in relevant media	Average diameter / Standard deviation	K-Kit / TEM		
	NOAAs	115 / 73 nm	(4wt% in DI water)		
	Nano-Objects	68 / 20 nm (number 58%)			
	Aggregations / Agglomerations	180 / 70 nm (number 42%)			
5	Solubility/Dispersibility	< 0.01% in Ca ²⁺ form	ICP/MS		
		Dispersed in DI water > 4 wt%	K-Kit / TEM		
		(20 ~ 450 nm)			
6	Surface charge	-23.4 ± 1.3 mV (in DI water)	Zeta potential		
7	Surface chemistry	Surface atom:	XPS		
		C (35%), O(48%), Ca(16%)			
8	Specific surface area	18.14 m ² /a	BET		



As Additive



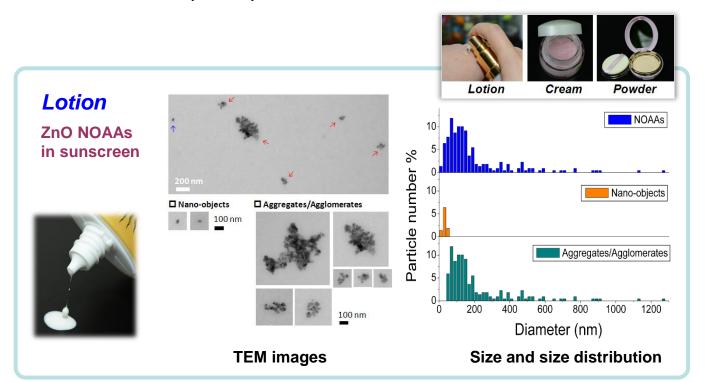
□ Size and size distribution of CaCO₃ NOAAs in milk





NOAAs of ZnO NPs in sunscreen

- K-kit can be used for characterizing NOAAs of cosmetics in final product forms, including lotion, cream, and powder, to assess the safety risks of nanomaterials in cosmetic ingredients.
- International Cooperation on Cosmetic Regulation Report (ICCR) 2012 Characterization of Nanomaterials II – Insolubility, Biopersistence and Size Measurement in Complex Media.
- European Union (EU) Cosmetics Regulatory (EC) No. 1223/2009
 Mandatory labeling of Nanomaterials as ingredients in Cosmetics (effective 2013/07/11)
- United States Food and Drug Administration Guidance (US FDA) 2012
 Guidance of Industry Safety of Nanomaterials in Cosmetic Products



Definitive:

Direct observation in final product form or relevant media, minimizing artifacts.

Quantitative:

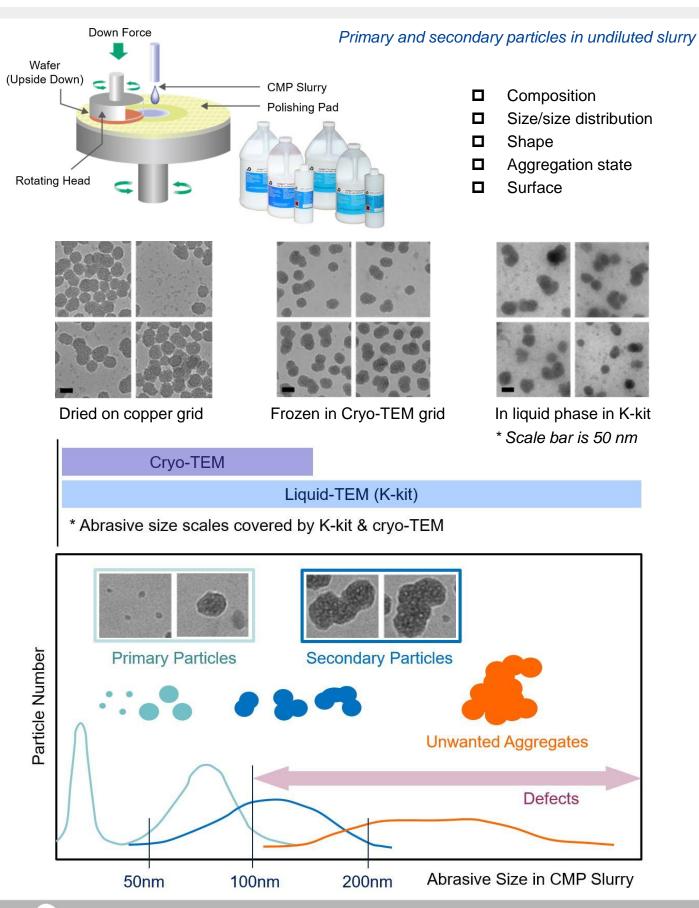
Image-based statistical analysis of aggregation and agglomeration as well as particle concentration.

Comprehensive:

BIO Ma-TEK

All physicochemical characterization requirements can be addressed.

K-kit Application: Abrasives in CMP Slurry

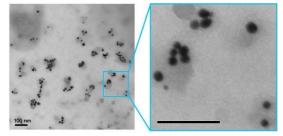


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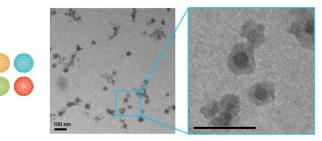
BIO Ma-TEK

K-kit Application: Liquid-TEM Observation in Nanopharmaceuticals

Applicable particle concentration for K-kit: 10¹¹~10¹⁴ particles/ml

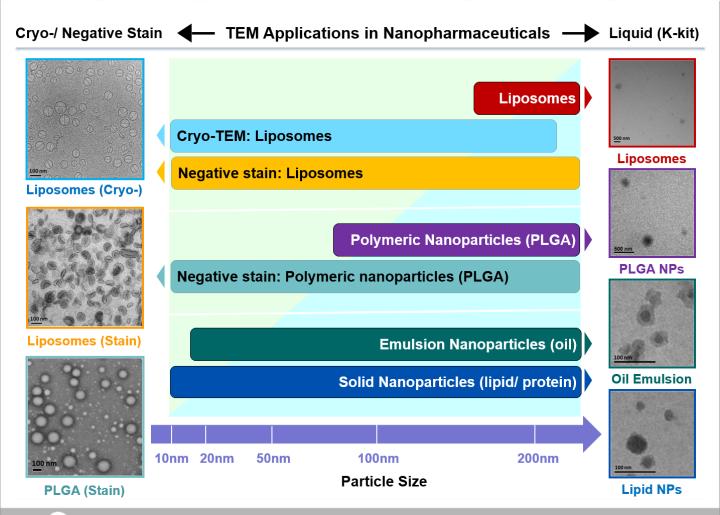


AuroVist® solution was directly loaded and sealed in a K-kit in liquid form.



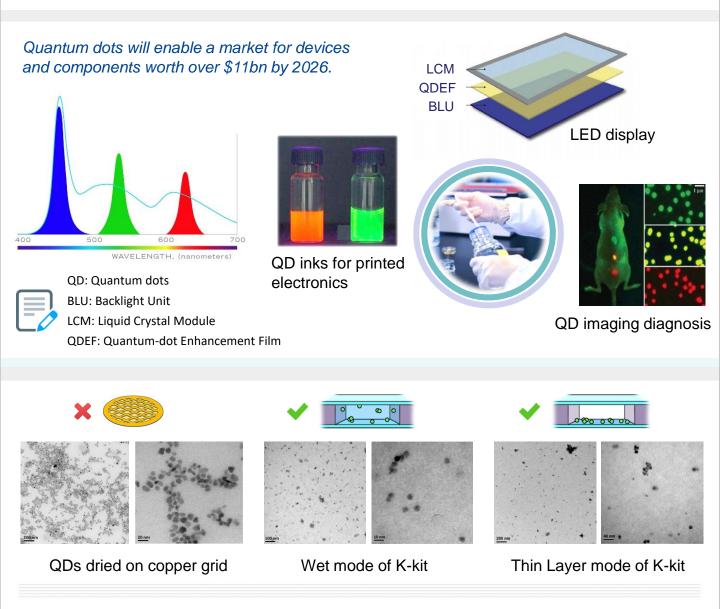
Oil emulsion in water was loaded and sealed in a K-kit in liquid form.

Brand Name of	Doxil ®	Abraxane ®	Aurimune ®	Resovist ®	Rexin-G ®
Pharmaceuticals	(1995 approved)	(2005 approved)	(Phase II)		(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	1.0 x 10 ¹⁴	4.3 x 10 ¹³	≦ 1.7 x 10 ¹²	1 x 10 ¹⁴	1-4 x10 ¹¹
Concentrations	liposome /ml	albumin particles /ml	gold particles /ml	particles /ml	cfu

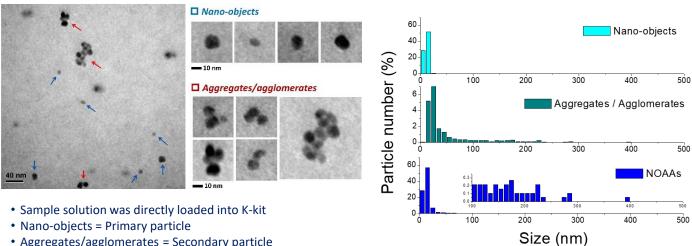




K-kit Application: Quantum Dots in Solution



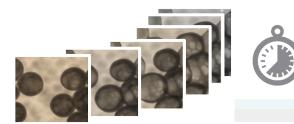
Size and Size Distribution of QDs in Chloroform (Thin Layer Mode of K-kit)



Aggregates/agglomerates = Secondary particle

BIO Ma-TEK

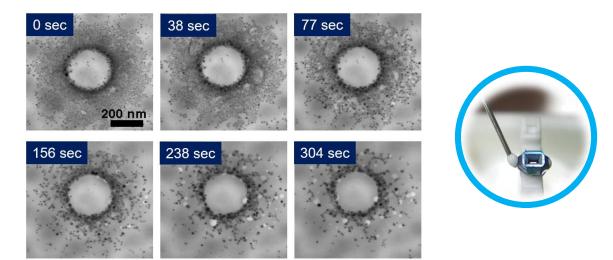
K-kit Application: In-situ Dynamic Observation



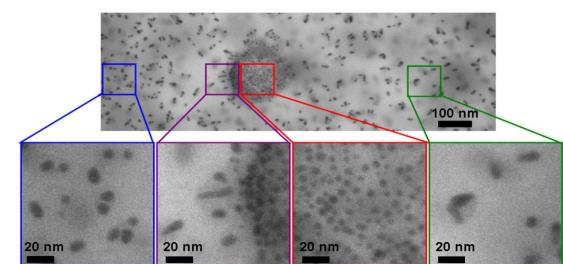
BIO Ma-TEK

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 the sodium ions, which induced from the TEM electron energy, in PBS buffer around a polystyrene bead could be observed with prolonged observation time.

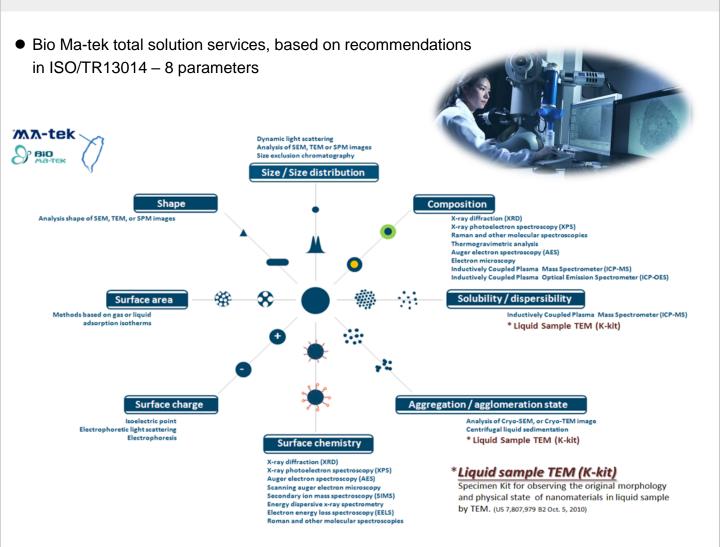


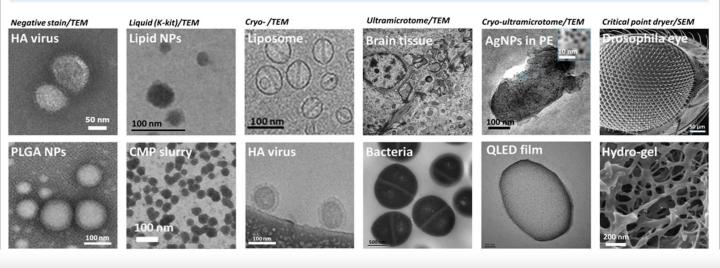
The metal growth of Au ions in water could be observed dynamically at the same time in the areas far away and nearby a polystyrene bead. (As shown in the image, there's a polystyrene bead at the center, with a lot of relatively small Au particles surrounded)

Gold metal growth in water with and without polystyrene beads

Not Only K-kit

Bio MA-tek provides comprehensive services in bio-EM and physicochemical analysis







Our Services



Bio MA-tek provides analytical services to the following industries:

- **Bio-technology** •
- Pharmaceutical (nano-formulation, nano-drug, etc.) •
- Vaccine (vaccine formulation, adjuvant, etc.)
- Medical devices (dialysis, dressing, etc.)
- Cosmetics (powder, cream, lotion, mask, etc.)
- Foods (additives, packaging materials, etc.)
- Academic & research organizations
- Electronics industry (Semiconductors, TFT-LCD, LED, PCBs, ...)
- Others

Service Items

Physico-Chemical Characterization

- Size/Size Distribution: DLS
- Surface Charge: Zeta potential

BIO Ma-TEK

- Composition and Impurity: TGA, DSC, FT-IR, XRD, UPLC, ICP-MS, LC/MS/MS (QQQ)
- Surface Chemistry: XPS
- Surface Area: BET

Electron Microscopy Analysis

- Negative Stain
- Resin Embedding
- Ultramicrotome
- Cryo-ultramicrotome
- Critical Point Drier (CPD)
- Cryo-transfer System
- Liquid Sample Preparation
- TEM/ EDX
- SEM/ EDX



sales@bioma-tek.com

Tel: +886-3-611-8611

Fax: +886-3-563-0777

project@bioma-tek.com

Bio Materials Analysis Technology Inc.



Established on March 31 2014, Bio Materials Analysis Technology Inc. (Bio MA-tek) and its sole investor, MA-tek, serve as the best R&D partners of high-tech industry. To address the demanding needs for physical and chemical characterization of nanomaterials in bio-medical industry. Bio MA-tek has introduced an array of bio-EM sample preparation and image analysis services as well as a comprehensive list of analytical services per the recommendations of ISO/TR13014.



Vision:

To become a leading brand in bio-medical materials analysis

Business model:

Focus on core technology, leverage external resources, deliver proficient and adequate services

Positioning:

Solution provider of nano- biomaterials characterization and analysis

Service scope:

Characterize and analyze nanobiomaterials in foods, cosmetics, medical materials, drugs, vaccines, biological tissue, etc. Provide proficient and adequate sample preparation, analysis, consultation, and contracted services.



The Best R&D Partner !







Bio MA-Tek, the Best R&D Partner for Your Success !



Distributor in USA

Structure Probe, Inc.

206 Garfield Ave West Chester, PA 19380-4512, USA

Phone 1-610-436-5400 1-800-242-4774

Bio Materials Analysis Technology Inc.

- Office: 1F, No. 26-2, Tai-Yuen St., Jubei City, Hsinchu County, 302, Taiwan
- Lab: 1A4, No. 1, Li-Hsin Rd. 1, Science-Based Industrial Park, Hsinchu City, 300, Taiwan