

1. 원료제조사에 대한 자료

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2. 원료에 대한 자료

1) 원료에 대한 설명 및 자료

(1) 원료의 특성에 대한 설명 자료 (상태, 구성성분 목록 및 함량 등)

① 상태 : 투명한 액상

② 구성 성분 : 정제수 (99%)+ Brewer's Yeast Extracellular Vesicles (1%)

③ 맥주효모세포외소포 판단 근거 :

NTA assay 결과, 농도와 사이즈가 세포외소포의 평균 농도($1-5 \times 10^{10}$)와 평균 사이즈인 **50-200nm** 사이로 확인됨.

Concentration : 3.2×10^{10} particles/ml

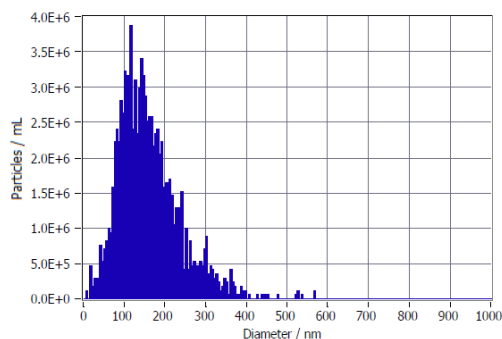
Mean size : 130.3nm

Median : 114.9nm

Concentration: 6.3E+7 Particles / mL
Dilution Factor: 500
Concentration Correction Factor: 1.00000
Original Concentration: 3.2E+10 Particles / mL

Result (sizes in nm)

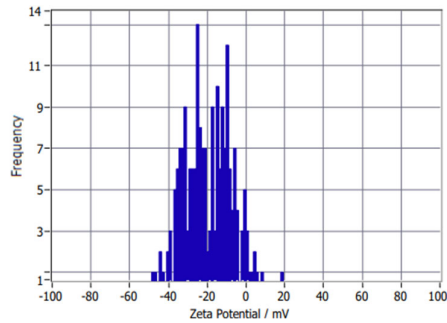
	Number	Concentration	Volume
Median (X50)	114.9	114.9	196.1
StdDev	54.7	54.7	109.8



	Number	Concentration	Volume
X10	77.5	77.5	110.7
X50	114.9	114.9	196.1
X90	191.9	191.9	396.2
Span	1.0	1.0	1.5
Mean	130.3	130.3	229.2
StdDev	54.7	54.7	109.8

Zeta potential 측정 결과, 세포외소포는 음(-)전하를 띄는데, 해당 성분에서 음의 (-44 ~ -10 mV)값이 확인 됨.

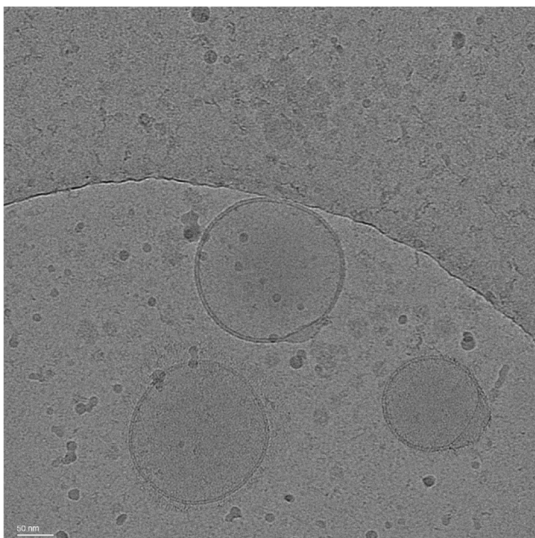
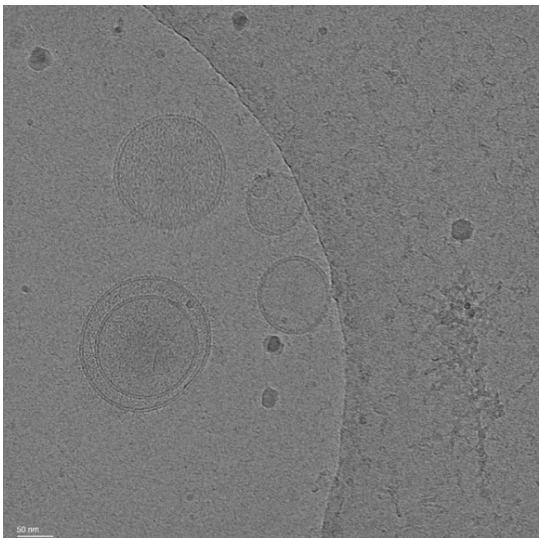
Zeta potential (Charge) : -20.403 mV



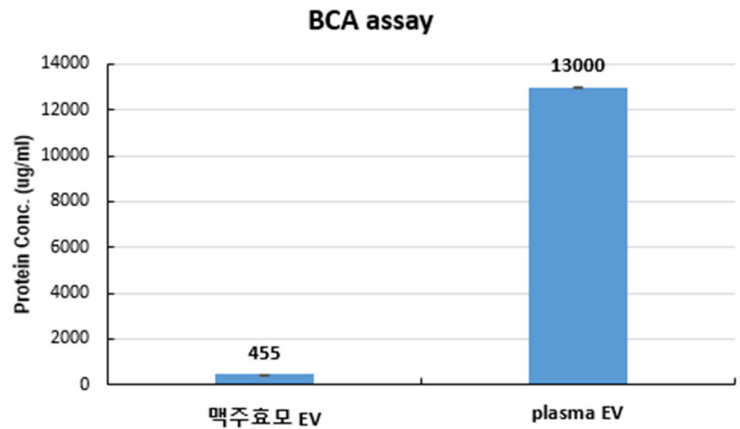
Stationary Layers	Zeta Potential / mV
SL1: 0.149	-20.403
SL2: 0.851	-18.518

Peak Analysis (Concentration)			
Zeta Potential / mV	Frequency	FWHM / mV	Percentage
-10.6	8.8	10.3	40.3
-24.5	9.5	5.1	30.0
-32.9	7.5	4.9	19.3
-0.4	3.4	4.2	8.5
-43.9	1.0	9.6	1.9

Cryo – TEM 촬영을 통해 인지질이중막을 확인하였음



맥주효모 EV 순도 : particle 농도가 비슷한 plasma EV (2.1×10^{10} particles/ml) 샘플에 비해 현저히 낮은 protein농도를 보이므로, 순도가 높은 EV 샘플임을 확인함



④ 원료의 효능 :

세포독성 실험에서 독성실험에 영향을 미치지 않는 맥주효모 엑소좀 농도 10%를 확인한 후, 진행한 실험임

- 상처치유 효과 : 맥주효모엑소좀 2.5-10%에서 양성대조군보다 더 높은 상처치유 효능을 보임

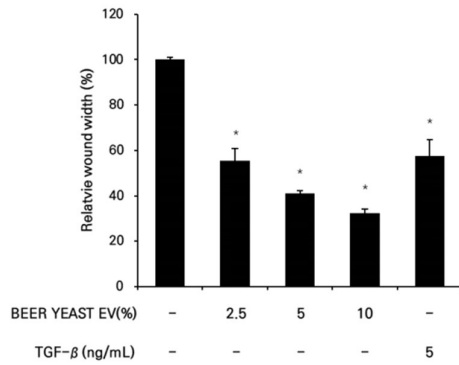


Figure 3. Change rate of wound width in HaCaT cells after treatment with 'BEER YEAST EV' (**P* < 0.05, negative control group)

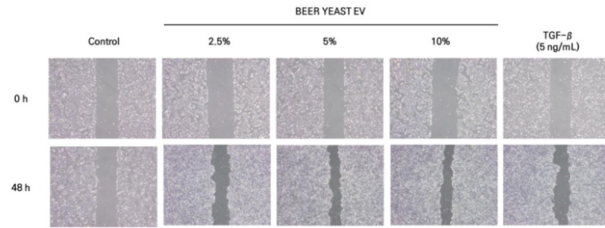


Figure 5. Cell migration images of HaCaT cells after treatment with 'BEER YEAST EV'.

- 콜라겐 생성 효과 : 맥주효모엑소좀 10%에서 가장 높은 프로콜라겐 생성효과를 보임

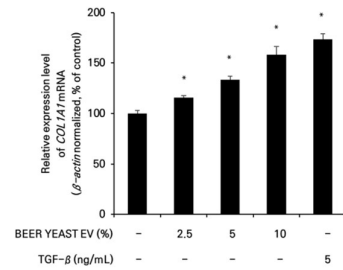


Figure 2. Measurement results of type 1 collagen (*COL1A1*) gene expression for 'BEER YEAST EV' in NHDF cells. (**P* < 0.05, negative control group)

2. Measurements of *COL1A1* mRNA Expression :

Relative expression values (%) of *COL1A1* mRNA normalized to *β-actin* expression

BEER YEAST EV (%)	-	2.5	5	10	-
TGF-β (ng/ml)	-	-	-	-	5
1 st measurement (%)	97.25	113.67	137.08	166.45	172.24
2 nd measurement (%)	102.74	116.04	132.07	157.52	168.93
3 rd measurement (%)	100.01	117.50	130.21	150.29	179.41
Average (%)	100.00	115.73	133.12	158.09	173.52
Standard deviation (SD)	2.74	1.93	3.55	8.10	5.36
.Student's <i>t</i> -test	1.00	0.00	0.00	0.00	0.00

- 항산화 효과 : 맥주효모엑소좀 50%이하 농도에서 항산화에 도움을 주는 것을 확인

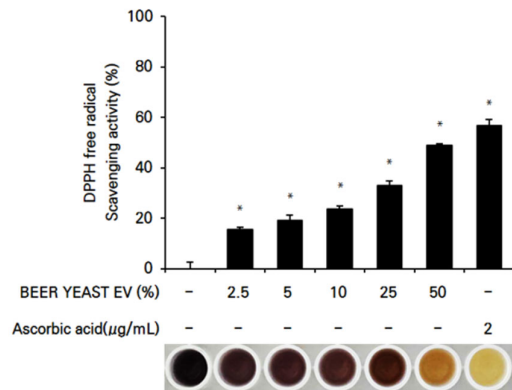


Figure 1. Measurement results of DPPH absorbance for 'BEER YEAST EV'.
(* P .05, negative control group)

- 항염 효과 : 맥주효모엑소좀 10% 이하 농도에서 항염 효능을 확인

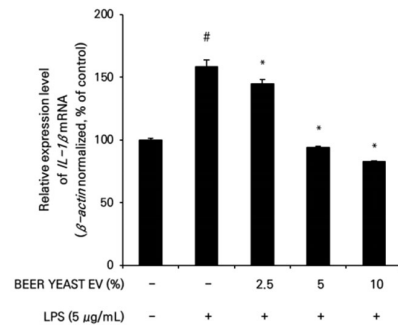


Figure 2. Measurement results of *IL-1β* gene expression for 'BEER YEAST EV' in LPS-induced HaCaT cells.
(# P .05, negative control group, or * P .05, LPS alone treated group)

2. Measurements of *IL-1β* mRNA expression :

Relative expression values (%) of *IL-1β* mRNA normalized to *β-actin* expression

BEER YEAST EV (%)	-	-	2.5	5	10
LPS (5 µg/mL)	-	+	+	+	+
1 st measurement (%)	101.16	163.37	145.94	94.10	83.45
2 nd measurement (%)	98.68	152.54	147.26	92.74	82.56
3 rd measurement (%)	100.18	159.64	140.75	95.02	82.78
Average (%)	100.01	158.51	144.65	93.96	82.93
Standard deviation (SD)	1.25	5.50	3.44	1.15	0.46
Student's <i>t</i> -test	1.00	0.00	0.00	0.00	0.00

(2) Manufacturing Procedure for Brewer's Yeast Exosome

① Preparation of Raw Material Extract

Brewer's Yeast Extract Preparation	1) Weigh 5 g of brewer's yeast powder. 2) Dissolve in 200 ml of purified water containing 4% glucose. 3)Mix using a roller shaker for 1 hour.
↓	
Extract Separation and Purification – Step 1	1) Centrifuge under the following conditions: 3,000 g / 4 °C / 15 min 2) Collect the supernatant. 3) Repeat centrifugation under the following conditions: 6,000 g / 4 °C / 15 min 4) Collect the supernatant.
↓	
. Extract Separation and Purification – Step 2	Filter the supernatant using a 0.8 µm filter (1st size filtration).

② Exosome Extraction (using ExoFilter – bottle-top)

Cationic Membrane Activation	1) Assemble the ExoFilter bottle-top vacuum filter, vacuum pump, and waste bottle. 2) Open the upper filter cap and add an appropriate volume of activation buffer (cationic membrane activator). 3) Operate the vacuum pump to filter the buffer completely into the waste bottle, then stop the pump.
↓	
Ion-Binding Based Exosome Capture	1) Open the upper filter cap and pour in the purified brewer's yeast extract. 2) Operate the vacuum pump to pass the solution completely into the waste bottle, then stop the pump. 3) Mechanism: Negatively charged exosomes bind to the positively charged membrane. Impurities <10 nm are removed by electrokinetic filtration.
↓	
Exosome Elution	1) Remove the waste bottle and replace with a collection bottle. 2) Add elution buffer (purified water with NaCl) at 1/20 of the original extract volume. → This step concentrates the sample. 3) Operate the vacuum pump until the entire buffer is filtered into the collection bottle, then stop the pump. 4) Exosomes detach from the cationic membrane and are recovered in the buffer.

↓

Buffer Exchange	1) Prepare a 50 nm TFF (Tangential Flow Filtration) unit with a peristaltic pump and feed tank. 2) Pour the eluted exosome solution into the feed tank. 3) Open the waste outlet and run the pump until all liquid is discharged, then stop. → Exosomes remain inside the TFF membrane. Impurities <50 nm are removed. 4) Close the waste outlet, add purified water (equal volume to the elution buffer) into the feed tank, and run the pump to recover exosomes. (Note: Exosomes can be used immediately after elution, but buffer exchange is recommended for final product quality.)
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③ Packaging and Storage

Packaging and Storage	1) Transfer the final exosome sample into a sterile storage vial. 2) Store in deep freezer at -80°C .
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2) 상품명 (영문) : Beer Yeast Extracellular Vesicles

3) 희망 INCI 명칭 (영문) : Brewer's Yeast Extracellular Vesicles

3. 기타 : 사업자 등록증 사본 1부 ← 첨부파일 확인